For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS

UNIVERSITY OF ALBERTA
QUAECUMQUE VERA

University of Alberta
Printing Department

Thesis
1966
#2D

THE UNIVERSITY OF ALBERTA

THE EFFECTS OF HYPERCAPNIA ON THE RENAL CIRCULATION IN DOGS

BY

Kenneth Norman Ackles

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA
JANUARY, 1966

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled THE EFFECTS OF HYPERCAPNIA ON THE RENAL CIRCULATION IN DOGS submitted by Kenneth Norman Ackles in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Acute hypercapnia was produced in two groups of chloralose anesthetized dogs with intact and denervated kidneys, by positive pressure ventilation with O_2 - CO_2 mixtures. I^{131} - Hippuran, creatinine and osmolar clearances and the I^{131} - Hippuran extraction ratio were measured and various parameters of renal hemodynamics were determined. In dogs with their renal nerves intact, at low Pa_{CO_2} (up to 70 mm Hg), filtration fraction (FF) increased while renal resistance (RR) remained unchanged, suggesting efferent arteriolar constriction and afferent arteriolar dilatation. RR increased at high Pa_{CO_2} suggesting efferent and afferent arteriolar constriction, with efferent constriction predominating (FF increased). At Pa_{CO_2} levels above 112 mm Hg decreased E_{Hipp} and the tendency towards the production of a more dilute urine suggest that cortical blood flow is decreasing relative to the

medullary blood flow.

In dogs with their left kidney denervated the pattern of response to hypercapnia was quite different, indicating that the renal nerves are necessary for the normal response of the kidney to hypercapnia. In the denervated kidney there was an immediate indication that increasing hypercapnia leads to shunting of the blood away from the cortex. This series of dogs also had a higher hematocrit than the dogs with the intact kidneys, so that changes in blood flow in the denervated kidneys due to viscosity changes are probably emphasized.

ACKNOWLEDGEMENTS

I am deeply indebted to Dr. C. Heath for his guidance and assistance with the experiments described in this thesis. Without his invaluable help, this work would have been impossible.

I would like to thank the Medical Research Council of Canada for financial support which made this work possible.

I would also like to thank Mr. Rene Roscher for his expert technical assistance in all experiments.

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION	1
LITERATURE REVIEW	3
Carbon Dioxide and the Kidney	4
Nervous Control of Renal Blood Flow	14
Carbon Dioxide and the Sympatho-Adrenal System	15
The Kidney and the Sympatho-Adrenal System	23
Renal Circulation	28
Renal Blood Flow	29
METHODS	45
Methods in General	45

Page

Methods in Detail 49

Anesthesia 49

Catheters 50

Control of Respiration 51

Urine Collection 52

Blood Sampling and Analysis 52

Hematocrit 53

Pressure Recording 54

Renal Denervation 54

Clearance Materials 55

Creatinine 56

I^{131}-Hippuran Determination 57

Osmolarity Determination 58

Computer Calculations 59

Statistical Methods 62

RESULTS 64

DISCUSSION 75

SUMMARY AND CONCLUSIONS 90

BIBLIOGRAPHY 93

TABLES 110

APPENDIX I - Preliminary Work 152

APPENDIX II - Abbreviations 157

LIST OF TABLES

Table No.		Page
1	Pa_{CO_2} Ranges	65
2	Extraction of Hippuran	110
3	Renal Blood Flow	114
4	Renal Plasma Flow	118
5	Effective Renal Plasma Flow	122
6	Glomerular Filtration Rate	126
7	Filtration Fraction	130
8	Urine Flow	134
9	Osmolar Clearance	138
10	Free Water Clearance	141
11	Renal Resistance	144
12	Hematocrit	148

LIST OF FIGURES

Figure No.		Facing Page
I	Breathing Circuit	51
II	Urine Fraction Collector	52
III	Fortran Program	59
IV	Data Printout	60
V	Results Printout	61
VI	Renal Plasma and Blood Flows	66
VII	Extraction of Hippuran	67
VIII	Renal Resistance	68
IX	Glomerular Filtration Rate	69
X	Filtration Fraction	70
XI	Urine Flow	71
XII	Osmolar Clearance	72
XIII	Free Water Clearance	73
XIV	Hematocrit	74

INTRODUCTION

Although the effects of increased carbon dioxide on renal hemodynamics have been studied for many years, observations made in the past have, for the most part, been incomplete, as total renal blood flow was frequently the only hemodynamic parameter measured. While it is generally agreed that increased carbon dioxide in the inspired air causes a decrease in total renal blood flow, there is no agreement on the intrarenal mechanisms responsible for this decrease.

Many preliminary experiments in which attempts were made to repeat the work of others in this field were discouraging because the methods and results did not satisfy our criteria for valid renal hemodynamic measurements, particularly with respect to minimal interference with the renal circulation. However, sufficient experience was gained with standard methods of renal physiology

to lead to the conviction that if enough variables were simultaneously measured, important new information would be obtained. By using this approach, it was hoped that the renal response to respiratory acidosis would be revealed in sufficient detail to indicate the intrarenal mechanisms involved.

LITERATURE REVIEW

Carbon dioxide - its production in the body, effects upon body systems, and elimination from the body - has been studied by physiologists for years.

Paul Bert, in his classical research published in "La Pression Barometrique" in 1878 (12), studied the effects of breathing high concentrations of carbon dioxide on the respiration and circulation in several species of animals. During the following forty years, several reports on the cardiovascular and respiratory effects of excess carbon dioxide were published such as that by Hill and Flack in 1908 (61), but these early workers often had difficulty distinguishing between the effects of carbon dioxide excess and oxygen lack.

The first definitive reports on the effects of carbon dioxide on the respiration and circulation of humans were by Schneider and Truesdell in 1922 (123), and Goldstein and DuBois in 1927 (52). Schneider and Truesdell had their subjects rebreathe an oxygen enriched mixture in which the oxygen content was maintained constant at 30%, while the carbon dioxide content was allowed to increase. They reported a gradual rise in blood pressure and heart rate with increasing carbon dioxide. Goldstein and DuBois obtained similar results which they plotted against alveolar carbon dioxide. However these workers (54) make no mention of any technique to prevent anoxia while rebreathing various carbon dioxide-air mixtures from Douglas bags. The early experiments on the effects of carbon dioxide on the cardiovascular system provide good descriptions of the gross changes observed, but usually they were not well controlled and many variables, such as pH and P_{CO_2}, could not be measured.

Carbon Dioxide and the Kidney

When a high concentration of carbon dioxide is breathed, urine production is greatly decreased. Many conflicting and confusing opinions regarding the basic mechanism behind this phenomenon are found in the literature. Part of the confusion results because different methods were used to produce the increase in carbon dioxide and these result in different concentrations of CO_2 at the various effector sites.

These methods were: (a) apneic oxygenation, (b) rebreathing, and (c) inhalation of gas mixtures containing carbon dioxide and oxygen.

(a) Apneic Oxygenation

Apneic oxygenation (diffusion respiration) has been defined by Krough (78) as "gas exchange between the atmosphere and the lung alveoli in the absence of rhythmically recurring differences in the barometric pressures of these two areas". This implies that respiratory movements of the chest are absent. Conversely, mechanical ventilation is accomplished by rhythmically recurring differences in pressure between the atmosphere and the lung alveoli. Because the respiratory quotient is less than 1, the pressure in the alveoli is less than atmospheric pressure and there is a continuous flow of air (or O_2) into the lungs. Therefore during apneic oxygenation, CO_2 is accumulated in the alveoli and hence in the blood, so that this is not an effective ventilation mechanism. However, Behnke and his associates (11) showed that apneic oxygenation occurs in the anesthetized dog under certain specialized conditions.

While studying the effects of four atmospheres of oxygen on anesthetized dogs, they (11, 122) noted that despite apnea, which is caused by

"oxygen toxicity", the blood remained well oxygenated for periods up to 30 minutes. Draper and Whitehead (40) found that at a barometric pressure of 630 mm Hg, apneic oxygenation could be accomplished if dogs were (a) denitrogenated, (b) had an open airway attached to an oxygen supply at atmospheric pressure, and (c) the circulation was intact. They used sodium thiopental to stop respiration and noted that dogs could survive with no respiratory movements for periods up to approximately two hours.

It was not until 1951, that observations on renal function were made during apneic oxygenation. Shires and Eyer (132) performed two series of experiments in which they noted renal changes. In the first series they allowed dogs, denitrogenated for one hour with 100% O_2, to rebreathe O_2 from a spirometer with its CO_2 absorber removed. In the second series, following denitrogenation, the dogs were given sufficient decamethonium bromide (Syncurine) to cause respiratory arrest. In the first series, it was observed that when the P_{CO_2} became high enough to cause apnea, anuria occurred at the same time. In the neuro-muscular blocked series, anuria also occurred simultaneously with the onset of apnea, but before the CO_2 level could have increased enough to be the cause. The origin of this apnea was further studied by denervating the left kidney, using the right kidney

as a "normal" control in the same animal. When this was done, urine flow remained constant in the denervated kidney but the intact kidney always showed complete anuria. Shires and Eyer (132) also demonstrated that bilateral section of the cervical vagus failed to prevent the anuria in the intact kidney. Hypotension and central nervous system depression were also ruled out as initiating factors for the anuria.

Many workers have confirmed the fact that anuria occurs at the onset of apneic oxygenation (thiopental induced) and also the fact that the anuria can be largely prevented by renal denervation (18, 75, 144).

Bohr et al (18) found that apneic oxygenation following a high dose of thiopental caused the clearance of PAH and creatinine to be depressed in both intact and surgically denervated kidneys, the depression being very much less in the denervated kidneys. They also observed a decrease in PAH extraction ratios, which appeared to correlate with the drop in blood pH. The decreased clearance of PAH in the denervated kidneys was found to be related to a fall in blood pressure. Since anuria occurs immediately after the beginning of the apnea, it is obvious that the CO_2 level could not have increased enough to be the prime stimulus causing the

anuria. It was postulated that the high dose of thiopental initiated renal vasoconstriction by a strong stimulus of central origin to the renal nerves (18, 141). This might be similar to the apneic renal reflexes observed by Bradley and Bing (21) who described a reflex reduction in renal plasma flow, glomerular filtration rate and urine flow in the diving seal, or the similar alterations in renal functions found during smoke apnea in rabbits by Forster and Nyboer (47).

Stone et al (141) studied apneic oxygenation occurring when thiopental or decamethonium prevented respiratory movement. They confirmed the previous observations (18, 75) that anuria coincides with the beginning of apnea when ventilation is stopped with thiopental, and concluded that this anuria is the result of a nonspecific renal vasoconstriction caused by central vasomotor activity. It is known that thiopental has both an anti-diuretic (60) and a central vasoconstrictor (59) action, so the immediate anuria is probably caused by the thiopental. Apnea also occurs coincidentally with the cessation of respiration after an intra-cisternal injection of procaine hydrochloride (66), which again seems to indicate centrally induced vasomotor activity.

When, on the other hand, apnea was induced by neuromuscular blockade (65, 141), it was observed

that urine production usually did not start to decline until fifteen minutes of the apneic period had elapsed. These findings are in direct contradiction to the results of Shires and Eyer (132), who reported that anuria and apnea began simultaneously when decamethonium was used to stop breathing. One possible explanation for these conflicting results, is that Shires and Eyer (132) used paraldehyde as an anesthetic, while other workers used light thiopental anesthesia.

The slowly developing oliguria, seen during apneic oxygenation induced by a neuromuscular blocking agent has been correlated with the gradually developing hypercapnia and acidemia (65). It is noted that the time course of the developing oliguria in animals made apneic by neuromuscular blockade (65) parallels closely the development of oliguria in the denervated kidneys of the dogs made apneic with intravenous thiopental (18, 75) or intra-cisternal procaine (66). If CO_2 is responsible for this gradually developing oliguria, its mechanism of action is difficult to explain on the basis of sympathetic activation. Although one might expect low concentrations of CO_2 to stimulate the sympathetic nervous system (7, 89), the threshold for the appearance of catecholamines in the blood has been shown to be a $C_{I_{CO_2}}$ of 15% (62, 145). Only the adrenal medullary hormones will be acting on the denervated kidneys of

the thiopental and procaine apneic series, while the adrenal medulla and the sympathetic nervous system will be acting on the intact kidneys of the neuromuscular block series. If carbon dioxide alone was responsible for the oliguria, it should develop more quickly in the innervated kidneys.

It has been demonstrated that when a buffer (THAM[1]) is infused during apneic oxygenation caused by neuromuscular blockade, in amounts sufficient to prevent a change in pH, a diuresis occurs. This diuresis is in contrast to the gradually developing anuria which is usually seen (69, 101, 102). Blood catecholamine levels never increase above normal if THAM is infused during apneic oxygenation (69). However, THAM is a strong osmotic diuretic (101, 110) and the fact that the anuria of apneic oxygenation is prevented, and is in fact turned into a diuresis by THAM, does not rule out the possibility that some factor other than acidosis is involved in the slowly developing anuria of neuromuscular block apneic oxygenation.

(b) Rebreathing

Few experiments have been performed in which renal function has been studied while increasing

---

[1] 2-amino-2hydroxymethyl-1,3-propanediol or tri (hydroxymethyl)-aminomethane.

inspired CO_2 by rebreathing. Shires and Eyer (132) did use this technique in one series of experiments but the only observation they report is that when apnea was produced from carbon dioxide intoxication, anuria followed immediately. They report that apnea and thus anuria occurred with carbon dioxide levels between 28 and 47% in the inspired air. The anuria was abolished by renal denervation.

Dowd et al (41) studied renal hemodynamics using standard PAH and creatinine clearance techniques, while increasing carbon dioxide by rebreathing. They found that during the first 150 minutes of rebreathing (CO_2 up to 16%), there was very little change detectable in effective renal plasma flow (ERPF). However, the glomerular filtration rate (GFR) progressively declined resulting in a decreased filtration fraction (GFR/ERPF). When a CO_2 content of 19-20% in the inspired air was reached, ERPF was reduced with no further reduction in GFR. They conclude from these results that renal hemodynamic changes appeared to be minimal until high CO_2 levels were reached and that there was no evidence of significant renal vasomotor alterations except at the highest CO_2 levels. Their results are questionable because at the highest carbon dioxide levels attained, the arterial CO_2 content was only 51.6 vol.% which corresponds to a P_{CO_2} of about 50 mm Hg which is not consistent with "high" inspired CO_2.

(c) Inhalation of Gas Mixtures of CO_2 and O_2

Several authors have studied renal function while their experimental animals inhaled gas mixtures containing carbon dioxide and oxygen.

Breathing low concentrations of CO_2 (5-7%) has been shown to cause a diuresis (8, 156). It is agreed that this diuresis is due to inhibition of the supraopticoneurohypophyseal system since it can be abolished by the administration of Pitressin (8).

The diuresis of hyperventilation is very similar to, but greater than the diuresis of negative pressure breathing (36). Hyperventilation with its characteristic intrathoracic pressure changes, is probably involved in the diuresis of low inspired CO_2. Valtin et al (156) conclude that the neurohypophysis is not being influenced by way of the left atrial stretch receptors which have been shown to cause a diuresis when stimulated by an increase in intrathoracic blood volume (60a). The only conclusions as to the mechanism involved, is that it is a non-osmotic influence on the supraopticoneurohypophyseal system (156). Higher concentrations of carbon dioxide than the above are known to cause an anti-diuresis and a reduction in renal blood flow.

Adolph, in 1935 (2), first noted a relationship between urine volume and carbon dioxide content of

the inspired air. He exposed frogs to an atmosphere of 30% carbon dioxide in oxygen and noted either a marked reduction in urine flow or complete anuria. He observed blood flow in the glomeruli directly, and reported that a reduction in glomerular blood flow was related to a decrease in urine volume. However, he observed that blood may be still flowing through glomeruli while urine flow has ceased completely.

Brassfield and Behrmann (22) allowed dogs to breathe various mixtures of CO_2 in O_2 (7-16%) spontaneously. They found that 16% CO_2 caused about a 50% reduction in urine flow, if the initial urine flow was high, and sometimes complete anuria, if the initial urine flow was lower. Their dogs attained an arterial pH of 7.08 on 16% CO_2.

Similar results were obtained by Franklin et al (48), who in addition to noting a decrease in urine flow, also noted a marked reduction of cortical blood flow. In their experiments, carried out on rabbits, the cortex was observed to change from a pink colour to white within seconds of the beginning of CO_2 inhalation. Many other workers have confirmed that a high concentration of carbon dioxide causes a reduction in renal blood flow. They used a variety of methods for the measurement of renal blood flow, e.g., direct measurement of renal venous outflow (142), PAH clearance (88), electromagnetic flow probe on the

renal artery (4, 24, 73) and an electromagnetic flow probe on the renal vein (24). It is also generally agreed that following renal denervation, minimal changes in renal blood flow occur on exposure of the animal to high concentrations of carbon dioxide (48, 73, 142).

Recently, Simmons and Olver (134) made small changes in acid-base balance in dogs (up to ± 0.2 pH units and one-half or double control Pa_{CO_2} levels). With an electromagnetic flowmeter on the left renal artery, they used the flows obtained to calculate renal vascular resistance (RVR). Increasing the P_{CO_2} always resulted in a decrease in RVR which was correlated with Pa_{CO_2} but not with pH.

A search of the literature has confirmed the statement of Kennedy (70) that "systematic and well controlled studies on the effect of CO_2 on renal perfusion, vascular resistance and glomerular filtration rate are not available".

Nervous Control of Renal Blood Flow

The existence of both vasoconstrictor and vasodilator fibres to the intrarenal vessels of the kidney has been recognized since the classical studies of Bradford in 1899 (20). In the dog, vasoconstrictor fibres to the kidney leave the cord in the anterior roots of segments T6-T12, most being concentrated in

T10-T12. These fibres, which are preganglionic, leave the sympathetic chain in the white rami by way of the lesser splanchnic and the lowest splanchnic nerves. The lesser splanchnic nerve receives fibres from T9-T10 and ends in the aorticorenal ganglion, while the lowest splanchnic nerve receives its fibres from the last thoracic sympathetic ganglion and terminates in the renal plexus (112). From the aorticorenal ganglion and the renal plexus, postganglionic fibres enter the hilum of the kidney, mostly in close association with the renal arteries. The function of the vasodilator fibres identified by Bradford (20), has not been determined. The intrarenal distribution of the vasomotor fibres is unknown. The nerve supply to the kidney is very profuse (20). Von Euler (158) has pointed out that the renal noradrenaline concentration is 3-4 times that of the liver and almost twenty times greater than skeletal muscle, which is an indication of a large number of sympathetic nerve endings in the kidney.

Carbon Dioxide and the Sympatho-Adrenal System

The autonomic nervous system is a very important element in the determination of the final response to a change in carbon dioxide environment. The nature of the system leads to complex reactions which are further complicated by direct effects of

carbon dioxide on the mechanism or organ under study. The sympathetic nervous system is activated by increased carbon dioxide in an orderly and progressive way, but antagonistic systems are also initiated which ensure a graded response. For example, an increase in carbon dioxide results in an excitation of the sympatho-adrenal system both by central nervous system pathways and by a less important direct action on the adrenal medulla. At the same time there are parasympathetic effects due both to central action and peripheral inhibition of acetylcholinesterase. The effector response to the increased level of circulating adrenaline in the blood is attenuated by the acidosis (or hypercapnia). However the adrenaline stimulates the anterior pituitary, which in turn brings about a release of adrenal cortical hormones which tend to restore the activity of the adrenaline.

Exposure of an animal to slightly elevated carbon dioxide levels (5-7%), causes the total peripheral resistance to increase. This is concluded from the fact that the blood pressure increases with little or no change in cardiac output. The heart rate usually increases slightly, depending upon the carotid sinus reflex. This cardiac acceleration is still seen after both vagi have been cut, but is not seen after section of the cardiac sympathetic fibres (89). Also, the blood pressure response to mild elevations in

carbon dioxide is completely abolished after sympathectomy (7).

Low levels of carbon dioxide have therefore been shown to activate only the sympathetic nervous system. The adrenal medulla does not participate in this response. An increase in blood catecholamine concentration has never been detected after exposure to low levels of carbon dioxide. Both Tenney (145) and Honig and Tenney (162) showed that the threshold level for the appearance of catecholamines in the blood is a concentration of 15% carbon dioxide in the inspired air. They also demonstrated that adrenalectomy in no way modifies the cardiovascular response to 6% carbon dioxide in the inspired air.

Higher concentrations of carbon dioxide initiate the release of catecholamines from the adrenal medulla, and probably from other sources of chromaffin tissue in the body. Early workers (30, 67) inferred from their observations of the effects of high concentrations of carbon dioxide on the cardiovascular system, that adrenaline was probably released by high levels of carbon dioxide. However, this could not be confirmed because bioassay methods failed to show any increase in circulating adrenaline (30, 50). This early failure to demonstrate an increase in adrenaline levels in the blood was probably due to either: (i) a

failure to reach a threshold concentration of carbon dioxide in the blood (50), or (ii) to a depression of sensitivity of the bioassay method, with the stimulating adrenaline effect being masked by the rising hydrogen ion concentration (64). However, when the denervated nictitating membrane was used as a sensing device, adrenal medullary response was shown to occur after six to eight minutes of apneic oxygenation (146). It was also observed when the alveolar carbon dioxide concentration was acutely increased to between 10 and 15% by breathing carbon dioxide mixtures (145). Using direct chemical methods, Richardson and Woods (119) detected increased blood catecholamine levels in dogs following breathing of 25% carbon dioxide. Similarly, Millar (93) measured increased adrenaline following 30 minutes of apneic oxygenation and also showed that the level of noradrenaline remained relatively low. It is interesting to note that a significant increase in adrenaline did not occur until the Pa_{CO_2} was over 175 mm Hg and the pH was below 6.8 (93).

It is generally accepted that the increase in Pa_{CO_2} and not the decrease in pH is the stimulus for the sympathetic-adrenal response. Tenney (145) observed that comparable adrenaline release was obtained from a stimulus of increased carbon dioxide whether or not sufficient bicarbonate was infused to

prevent a decrease in blood pH. Also, several authors (37, 58, 67) have observed that the cardiovascular response to CO_2 is more dramatic than the response to a comparable level of metabolic acidosis.

The location of the receptor site which responds to increased carbon dioxide in terms of the cardiovascular response is still not completely settled. The adrenal medulla is stimulated directly only with very high levels of carbon dioxide (147). In dogs, a maximum cardiovascular response to increased CO_2 is seen only with intact medullary centers (30).

Further evidence that the cardiovascular response to increased carbon dioxide is due primarily to activation of the sympathetic nervous system and not to direct stimulation of the adrenal medulla is given by the experiments of Honig and Tenney (62) and Mathison (87). They showed that the cardiovascular response to carbon dioxide is almost completely depressor if the spinal cord is destroyed. Tenney (145) found that only with very high levels of CO_2 (30%), was there evidence of an adrenal medullary response in an animal with its spinal cord destroyed.

Carotid and aortic chemoreceptor areas are of particular importance in the cardiovascular responses to CO_2 in the dog (62). These responses are mediated by the sympathetic nervous system (147). In 1935, Samaan and Stella (121), while recording

from a branch of the sinus nerve containing no baroreceptor fibres, showed an increase in chemoreceptor activity if the arterial pCO_2 was increased above 35 mm Hg. Others have extended these studies and obtained pure chemoreceptor preparations in which they showed some chemoreceptor activity at CO_2 tensions below 30 mm Hg (159).

Sympathetic nervous system excitation and adrenal medullary discharge are the most important mechanisms behind the cardiovascular response to hypercapnia. However, the overall response observed is attenuated by the acidosis and hypercapnia, even though these are the stimuli which evoke it. Part of the attenuation of the cardiovascular response to hypercapnia is due to activation of the parasympathetic nervous system. This parasympathetic activation is probably both central and peripheral. Young et al (165) and Bohr and Helmendach (17) reported increased vagal activity with increasing carbon dioxide levels. When the difference in heart rate response to hypercapnia is compared in intact and vagotomized dogs, Mithoefer and Kazemi (94) conclude that about 60% of the bradycardia response to hypercapnia is centrally mediated with vagal transmission, and what remains of the response is either still of central origin with a different transmission path or is mediated peripherally. The fact that the spinal dog responds in the same way as does the vagotomized animal indicates

that the remaining response is probably peripheral (94). It has been shown that carbon dioxide, through its acidifying effect, decreases the velocity of acetylcholine hydrolysis by acetylcholinesterase (100). This would result in a prolonged action of any quanta of acetylcholine released, resulting in an increase in parasympathetic activity.

Another factor which attenuates the sympatho-adrenal response is the reduced effect of adrenaline and noradrenaline on the cardiovascular system during hypercapnia. This effect, which has been seen by many workers on several systems, is thought by some to be entirely dependent upon the acidosis and not the increased carbon dioxide (120, 138, 145). However Bygdema and von Euler (29), Tobian et al (152) and Nash et al (104) favour the viewpoint that with respect to vascular smooth muscle, the increase in carbon dioxide is more important than the decrease in pH in the attenuation of the vasoconstrictor response to noradrenaline. Nash et al studied the reduced response of isolated vascular smooth muscle to noradrenaline during periods of high P_{CO_2} in the bath medium. They studied the site of action of this hypercapneic depression and concluded that it acted at some intermediate stage between the drug-receptor

and the contractile mechanism, perhaps the coupling reaction between the receptor and the contractile mechanism. They postulate that high carbon dioxide may depress the responses to submaximal doses of noradrenaline by influencing the availability of calcium for the contraction. This could perhaps explain the attenuation of the sympathetic nervous system response to hypercapnia as noradrenaline is thought to be the sympathetic post-ganglionic transmitter substance.

The sympathetic response to hypercapnia is probably somewhat attenuated by the high carbon dioxide. However, once the carbon dioxide level is high enough to stimulate adrenal medullary secretion, other defence mechanisms are mobilized to combat the attenuation of the cardiovascular response to adrenaline. This mobilization is the result of the increased adrenaline, and not the direct effects of high carbon dioxide on the adrenal cortex (118). The anterior pituitary secretes ACTH either by direct action of adrenaline on the anterior pituitary (84), or by a combination of a direct and neural activation by way of the hypothalamus (98), although the exact mechanism is not clear. When the carbon dioxide level is high enough to stimulate the adrenal medulla, the adrenal cortex is also activated. This is probably part of the general response of the

animal to a stressful situation. The result of this increase in adrenal cortical hormone level is that the threshold for the action of adrenaline is lowered (147), thus reducing the attenuation produced by the hypercapnia on the adrenaline response.

Probably of greater importance in the increase in smooth muscle responsiveness to adrenaline is the large increase in serum potassium (16). Serum potassium rises during acute exposure to high concentrations of carbon dioxide (25, 85). In addition, partly due to a direct carbon dioxide effect on the hepatic cells, but mostly through the liberation of adrenaline, high concentrations of carbon dioxide release glucose from the liver (46). Potassium moves with the glucose and accounts for the observed rise in serum potassium. If the carbon dioxide level is below the threshold for adrenal medullary activation, the serum potassium usually decreases (46). The increased potassium is thought to increase the response of smooth muscle to adrenaline (27, 49).

The Kidney and the Sympatho-Adrenal System

It has been well established by many investigators (3, 35, 63, 139) that adrenergic stimuli such as renal nerve stimulation or administration of adrenaline produces intense vasoconstriction

of the renal vasculature. The precise site or sites along the complex renal vascular network which participate in adrenergic vasoconstriction have yet to be determined conclusively.

Using isolated dog kidneys, Worthen et al (164) concluded that the primary vascular effect of adrenaline is presumably on the afferent arterioles. When they infused adrenaline they found no consistent diuresis, no increase in kidney weight, a decrease in blood flow, and a decrease in GFR. Moyer and Handley (96) agree that adrenaline acts primarily on the afferent arteriole.

Conversely, the efferent arteriole was said to be the site of adrenaline action by Winton (163) and Richards and Plant (117), both sets of workers using perfused kidneys.

The effects of noradrenaline infusion were studied on intact dog kidneys by Blake (14), and no evidence was found for either afferent or efferent arteriolar constriction since no change in GFR was observed. From his observations (a decrease in free water clearance) Blake concludes that noradrenaline may augment medullary flow at the expense of cortical nephrons.

During adrenaline infusion in dogs, Aukland (5) found that the effective renal plasma flow (C_{PAH}) decreased markedly, while GFR remained unaltered or

decreased slightly. Noradrenaline, on the other hand, had no consistent effect on C_{PAH} while GFR remained unaltered or increased slightly.

Smith et al (137) reported that adrenaline administration in man brought about a prominent increase in renal venous resistance. They proposed that venous constriction increased peritubular capillary pressure which would in turn decrease the reabsorption of tubular fluid and result in renal swelling. Zimmerman et al (166) recently reinvestigated the contribution that the renal venous resistance makes to the total renal resistance change seen with catecholamines. They conclude that the arterial side of the kidney possesses a much greater constrictor capacity than does the venous side and that renal vasomotor adjustments are made mainly on the arterial side of the vascular bed. It is postulated that the renal venous resistance changes which do occur are due to renal "effluent" constrictions at the arcuate-interlobar vein boundary (166). Anatomical evidence for such structures has been described by Koester, Locke and Swann (74).

De Maria et al (39) found that an intravenous injection of adrenaline caused an intense constriction of the renal arteries proximal to or at the first major bifurcation as visualized with angiography and cineradiography. In one-quarter

of the dogs, the renal artery constriction was noted to occur within one to one and one-half centimeters of their aortic origin. The renal arteries proximal to the constricted zone were dilated. Although they call their dose of adrenaline "pharmacological", they state that similar changes have been observed in humans who suffered from acute apprehension during the test period.

Brody and Fischer (23) employed a technique similar to that of De Maria et al (39) but perfused the kidneys at constant flow. They compared the effects of direct renal nerve stimulation with an infusion of catecholamines administered at a rate sufficient to produce an increase in perfusion pressure equal to that produced by the renal nerve stimulation. Electrical stimulation of the renal sympathetic nerves produced vasoconstriction of the larger renal vessels, i.e., the major renal and interlobar arteries. Similar findings in rabbits have been reported by Trueta et al (153), although the stimulation used by these workers was far more intense than that used by Brody and Fischer (23). The implication of these results is that a considerable portion of the total resistance change induced by nerve stimulation in the kidney may occur at sites other than the small vessels. However, adrenaline or noradrenaline infusions into the renal arteries (23),

caused dilatation of the renal and interlobar aretries, as opposed to constriction noted with the nerve stimulation. This dilatation was undoubtedly passive in nature and probably occurred as a result of intense vasoconstriction downstream in the smaller arterial segments (23). Similar observations for the catecholamines have been reported by Voudoukis and Boucek (160).

It is interesting to speculate why the effects of noradrenaline released from the nerve terminals should be different from noradrenaline circulating in the blood. Since Abrams et al (1) in dogs, and Trueta and associates (153) in the rabbit, have shown that large doses of catecholamines are capable of constricting the large intrarenal vessels, it may be that the lack of effect on large vessels seen by Brody and Fischer (23) results primarily from inability of the catecholamines, in low, but otherwise effective concentrations, to diffuse to the receptor sites. A second possible contributing factor to the above difference might be that the infusion of noradrenaline has a more profound effect on venous segments than does nerve stimulation as is suggested by the study of Zimmerman et al (166).

Regardless of the mechanism responsible for the different patterns of intrarenal vasoconstriction obtained by catecholamine infusion and

nerve stimulation, it is suggested that the intrarenal hemodynamics may be controlled by the sympathetic nervous system in two ways. Reflex activation of the renal sympathetic nerves might be expected to constrict the larger vessels within the kidney, whereas catecholamines released from the adrenal medulla, and carried in the blood, might be expected to affect primarily the small vessel segments.

Renal Circulation

The following brief description of the anatomical aspects of the renal circulation is taken from Selkurt (126).

The renal artery divides into interlobar arteries which subdivide into primary, secondary and tertiary arcuate arteries from which spring interlobular arteries. The afferent arterioles arise from these. In the dog, the afferent arterioles usually supply one glomerulus, but rarely may branch to supply two to four glomeruli, with a total of 200,000 glomeruli per kidney.

The glomerular capillaries converge on the efferent arterioles, then branch into a second capillary bed around the convoluted tubules, which drains into the interlobular veins. These lead into the arcuate veins, then the interlobar veins, and finally into the renal vein.

The glomeruli and associated tubules which lie deep in the cortex adjacent to the medulla (juxtamedullary glomeruli) have distinctive modifications of their circulation compared with the cortical glomeruli and nephrons. The juxtamedullary glomeruli are typified by long loops of Henle dipping into the medullary zone and papillary portions of the kidney, and have associated modified vascular structures. The efferent arterioles from these glomeruli not only break up into the typical capillary supply to the convoluted portions, but also form long hairpin loops of thin-walled blood vessels, the vasa recta, which accompany the loops of Henle. Although thin-walled, their diameter is several times greater than that of the typical peritubular capillary.

Renal Blood Flow

The accurate measurement of renal blood flow is very difficult. At the present time several methods of measurement are used.

(a) Total Blood Flow Measurement

(i) Direct Measurement

Renal blood flow has been measured directly many times. The usual appraoch is to cannulate the renal vein either directly (125), or by means of a long cannula passed from the jugular vein, into the

inferior vena cava and then into a renal vein (91, 103). The renal venous outflow is then measured directly either by a timed collection into a graduated cylinder (128), or it is run through a flowmeter (91, 103, 125). In recent years, the noncannulating electromagnetic flow meter applied to the renal artery (24, 73, 92, 128) and the renal vein (24) has been very useful in the measurement of renal blood flow.

(ii) Indirect Methods

Several indirect methods are available for the measurement of the total renal blood flow. In 1953, Conn et al (34) adapted the indirect Fick inert gas method for the kidney, which was first developed by Kety and Schmidt (71) for the measurement of cerebral blood flow with nitrous oxide. The ease of analysis was subsequently increased when the radioactive gas Krypton^{85} was substituted for nitrous oxide (26, 51). Recently, this method has been critically evaluated by Renner et al (113). In general, errors result because the very small arteriovenous difference in concentration of the inert gas make it difficult to determine accurately the time of final equilibrium between arterial and venous concentration.

A very interesting and apparently accurate method has recently been developed by Aukland and his associates (6). This method measures the rate of H_2

desaturation of the kidney using two catheter electrodes, one situated in an artery and the other in the renal vein.

(b) Intrarenal Blood Flow Distribution

(i) Miscellaneous Methods

Much interest during the past decade has been focused on the distribution of blood flow within the kidney.

Measurement of separate cortical and medullary blood flow offers many technical and/or theoretical difficulties. The method of Kramer, Thurau and Deetjen (77) is one of those which is technically difficult. They calculated regional blood flow by means of local blood volume and local circulation time. Circulation times of blood in the cortex and the outer and inner medulla were taken from dye-dilution curves in these areas, recorded simultaneously after a single injection of Evans blue or Cardio green dye into the renal artery. Recordings were taken by small photoelectric probes placed on the cortical surface and on one side of the papilla or further up into a calyx. In the cortex, a photoelectric reflectometer was pierced under the capsule, while the medullary photocells received light transmitted from a small tungsten bulb at the end of a hypodermic needle pierced

through the tissue. The results of this method give values in terms of total blood flow as follows: cortex 87.8%, outer medulla 11.3%, and inner medulla 0.9% (77, 154).

An excellent method has been developed by Thorburn et al (149), making use of the disappearance of Kr^{85}. In contrast to other methods using Kr^{85} (26, 51) which require both renal arterial and renal venous catheters, and the collection of serial blood samples, Thorburn's method requires only the catheterization of a renal artery with no blood sampling. After a single injection of Kr^{85} into the renal artery, the disappearance curve of the gamma emmission is monitored with a scintillation probe placed on the body surface above the kidney. The decay curves obtained in the kidney were nonexponential in contrast to other organs, but they could be described by a series of exponential curves, each associated with different blood flow rates through localized regions of the kidney. Four regions could be differentiated: (1) cortex, (2) juxtamedullary cortex and outer medulla, (3) inner medulla, and (4) perirenal and hilar fat. Because the removal of a highly diffusible substance such as Kr^{85} is affected by the counter-current exchange mechanism between the descending and ascending limbs of both the vasa recta and loops of Henle, flow values for inner and outer

medulla are probably lower than the actual values. However, using this method, the values obtained are of the same order of magnitude as those found by Kramer et al (77). Average blood flows as calculated by this method are as follows: compartment I (renal cortex) 73.5%, compartment II (juxtamedullary cortex and outer medulla) 20.6%, compartment III (inner medulla) 2.6%, and compartment IV (hilar and perirenal fat) 3.3%.

Lilienfield et al (81) measured the rate of accumulation of I^{131}-albumin in the renal papilla. Although their average value for medullary blood flow agreed well with that reported by Kramer et al (77), their method has several disadvantages. The data are extremely variable with a wide spread, and the method necessitates the removal and freezing of the kidney so duplicate determinations on the same kidney are impossible.

Ochwadt (105) fractionated renal blood flow into three compartments by measuring the rate of washout of blood containing Cr^{51}-tagged red cells and I^{131}-albumin. This method again gives values for the intrarenal distribution of blood flow that agree with those of previous workers (77, 81, 149) but is applicable only to isolated kidneys in which recirculation can be prevented.

The dye-dilution technique was used

by Reubi et al (115) and by Cohn and Combos (33) for renal blood flow measurement in man. They made a single injection of a known quantity of dye (Evans blue or Cardio green) into one renal artery and recorded the dye concentration from the renal venous blood. The last part of the dye-dilution curve showed a nonexponential slope which, according to Reubi et al (115), can be used to calculate the medullary blood flow rate. However, this calculation is sometimes difficult because recirculating dye affects the last part of the dilution curve.

(ii) PAH and Hippuran Clearance and Extraction

In the mammalian kidney, secretion of an organic compound can be attributed to one of two major systems of the proximal tubule, the organic acid (or hippurate) system (140) and the organic base system (107). This assignment of compounds into one or the other system is based largely on experiments demonstrating "competition" for secretory pathways. If two compounds are secreted, and if each can depress the secretion of the other, this is usually interpreted as indicating competition for a common secretory mechanism. So many diverse compounds are transported by the organic acid tubular secretory mechanism that it has not been possible to define any minimal structural requirement other than the compound must be an organic

acid (162). It has been established that para-aminohippuric acid (PAH), sodium hippurate and o-iodohippurate all share the organic acid excretory pathway (43, 57, 136, 162).

The term "clearance" was first used in connection with the excretion of urea by Möller, McIntosh and Van Slyke in 1929 (95). They defined it as "the volume of blood that one minute's excretion of urea suffices to clear of urea (UV/B)". Since all the blood flowing through the kidney is partially cleared of urea, this is a virtual rather than a real volume. No attempt was made to explain urea clearance in terms of any particular process in the kidney (95). Since 1931, when Jolliffe and Smith (68) extended the concept of clearance to the excretion of creatinine, it has been widely used to describe the excretion of other substances. If the concept of clearance is used to measure glomerular filtration, i.e., the volume in ml/min which is filtered through the glomeruli, the substance selected must fulfil certain specifications (135):

1. It must be completely filterable at the glomerulus.
2. It must not be synthesized or destroyed by the tubules.
3. It must not be reabsorbed or secreted by the tubules.
4. It must be physiologically inert.

5. It must be capable of being determined accurately in the plasma and urine.

Inulin has been shown to fulfill these specifications in the dog (130) and man (129), and creatinine does in the dog (130). The clearance of these substances is therefore said to measure the glomerular filtration rate in these species.

The fact that the clearance of a substance is less than the simultaneously measured inulin clearance is taken as evidence that the substance is undergoing tubular reabsorption. Similarly, if the clearance is greater than the inulin clearance, the substance is said to be undergoing tubular secretion, as long as the substance is not being synthesized by the kidney (135). After examining the clearance of a great number of substances, Smith and his associates (136) found that certain organic acids, e.g., PAH and phenol red, had identical and maximal clearances with respect to the inulin clearance.

If plasma is completely cleared of a substance in one passage through the kidney, then the clearance of that substance would be a measure of the renal plasma flow and its extraction ratio (131), (A-V)/A, would be 1.0. While no substance has been discovered having an extraction ratio of 1.0, the clearance of organic acids such as phenol red, diodrast, hippuran and its derivatives, which

have extraction ratios up to 0.9, are said to measure the "effective renal plasma flow", that is, the renal plasma flow which perfuses functional renal tissue. It is Smith's opinion (135) that in man, with an extraction ratio of 0.9 for diodrast or hippuran, that the 10% of the total blood flow not cleared of these substances represents the flow to non-functional renal tissue such as the capsule and perirenal fat.

In 1958, using stop-flow techniques, Malvin et al (86) found that the site of para-aminohippuric acid (PAH) secretion was confined to the proximal convoluted tubule only. Previously, Taggart (143) had demonstrated that when kidney slices were suspended in a solution containing PAH, the accumulation of PAH occurred only in the cortex, slices of medulla being completely inactive. On this basis, in 1958, Reubi (114) proposed that not only total renal plasma flow but also the distribution of plasma flow within the kidney could be approximated from the clearance and extraction of PAH.

Reubi proposed that the incomplete extraction of PAH results from an admixture of two flows; the clearing or cortical flow, from which PAH is 100% extracted, and the nonclearing or medullary flow from which no PAH is extracted. The cortical flow includes flow to proximal convolutions of both

cortical and juxtamedullary nephrons, while medullary flow includes flow to loops of Henle, the collecting ducts and inert tissue. Basic assumptions are that only the convoluted portions of the proximal tubules secrete PAH and that the cortical interstitial fluid constitutes a single well mixed compartment (109).

In the normal dog, the renal extraction ratio of PAH, while variable often lies around 0.85 (108). According to Kramer's photoelectric measurements (77), the cortical blood flow in the dog is about 85% of the total blood flow, while 10-15% flows through the outer zone of the medulla and 1-2% through the inner papillary zone. These figures seem to support the hypothesis that under normal conditions the blood flowing through the renal cortex is entirely cleared of low concentrations (below Tmax) of PAH, while the PAH content of the medullary blood remains unchanged (114). If this assumption is correct, the PAH extraction ratio should depend on the ratio between cortical and medullary flow. An increase in medullary flow without change in cortical flow or a greater increase in medullary flow will decrease the extraction ratio. Similar changes in the extraction ratio will be elicited by a decrease in cortical flow without change in medullary flow or a greater decrease in cortical flow. Changes opposite to these would result in

an increased extraction ratio.

Smith and his associates (88, 135), on strictly anatomical grounds, argue that in the outer medullary zone the vasa recta are intermingled with the descending straight limb of the proximal tubule, which is probably also capable of removing PAH from the vasa recta. They therefore believe that PAH is extracted from medullary blood as well as from cortical blood, and that the uncleared fraction ($1-E_{PAH}$) represents blood supplying the renal pelvis, capsule and perirenal fat.

According to Thurau (150), an "arterial" concentration of PAH in the medullary blood is a prime requirement for Reubi's hypothesis (114) but micropuncture studies of PAH concentration in vasa recta blood failed to verify this assumption (124). In golden hamsters with arterial PAH concentrations between 1.2 and 10 mg% and renal extraction ratios up to 0.82, the PAH concentration ratio of vasa recta plasma/arterial plasma during antidiuresis is considerably higher than 1.0, ranging between 4 and 12. During osmotic diuresis (mannitol and NH_4Cl), this ratio ranged between 1.1 and 2.5 (74). Thurau (150) states that even though a counter-current diffusion of PAH may exist in the vascular loop, a net influx of PAH from other sources must be postulated. This

is most readily explained by assuming diffusion out of the loops of Henle and collecting ducts, in which PAH concentrations are high. Accordingly, renal cortical plasma flow is underestimated and renal medullary plasma flow is overestimated by the Reubi technique. However, the marked decrease in vasa recta plasma/arterial plasma concentration ratios in osmotic diuresis implies either a very efficient counter-current accumulation in hydropenia or a very marked increase in papillary blood flow in osmotic diuresis (109). Pitts and his coworkers (109) think that it is by no means certain that one may safely extrapolate findings on the golden hamster to the dog. The uncertainties inherent in the assumptions involved emphasize that the distribution of blood flow between cortex and medulla calculated by the Reubi method are at best approximations and that changes in distribution induced by experimental variables are of more significance than absolute values of flow (109).

However, several workers have experimental evidence which supports Reubi's hypothesis. It has been observed many times that a low extraction ratio of PAH is associated with a low hematocrit and that an infusion of red cells results in an increase in extraction ratio (72, 116, 148).

Reubi et al (116) explains these observations with a postulate of cortical vasoconstriction in anemia which is relieved by restoration of a normal hematocrit. They also say that the difference in blood viscosity before and after a red cell transfusion contributes to the difference in cortical and medullary flow distribution.

Selkurt and Elpers (128) studied the simultaneous E_{PAH} and osmolar clearance during hemorrhagic shock in dogs, and noted a decrease in E_{PAH} which corresponded to a decrease in the concentration of the urine. They explain this as evidence of a relative increase in medullary blood flow which results in a washout of the osmolar gradient in the medulla.

Selkurt (127) found that while total renal blood flow increased during 30 minutes of ureteral blockade in dogs, the extraction of PAH always decreased. Selkurt (127) believes that the close correlation between the decrease in E_{PAH} and the increase in blood flow indicates the opening of A-V shunts. The increased flow in such pathways, which bypass active secretory tissue, would be reflected in a decrease in the A-V difference of PAH concentration in direct proportion to an increase in flow through such a circuit. He further believes

that the observed effect involves an increased circulation through the medullary zone, probably the vasa recta. This is supported by previous experiments where a simultaneous loss of renal concentrating power was observed (128).

Gömöri et al (53, 54, 55, 97) correlated the decrease in PAH extraction found during different kinds of shock with the results of studies of renal vascular casts, to indicate opening of shunts within the juxtamedullary region and the cortex. Those in the cortex appear to bypass tubules completely.

The intravenous infusion of human (53), bovine or canine (44) serum albumin into dogs, was observed to result in a decrease in the extraction of PAH. Since no change in Tm_{PAH} was observed, it was concluded that the decrease in E_{PAH} was not due to an impairment of tubular function but rather to the opening of medullary shunts within the kidney.

Lilienfield and Braun (80) observed a decrease in E_{PAH} during 20% mannitol administration. The decrease in E_{PAH} was accompanied by an increase in total plasma flow and a decrease in GFR. These changes are interpreted to indicate that mannitol dilates the efferent arterioles of the juxtamedullary glomeruli and permits increased shunting of blood to the medulla.

Earley and Friedler (42) explain the decrease in E_{PAH} which they observed during saline loading in the dog by an increase in medullary blood flow.

Reubi's hypothesis relating E_{PAH} to medullary blood flow was tested by Pitts and his associates (109). They found no change in E_{PAH} when plasma PAH concentration was increased from low up to, but not exceeding, Tm levels. If the plasma perfusing the cortex is completely cleared of PAH in one circulation through the kidney, then increasing the plasma level of PAH should not decrease the extraction ratio of PAH. They found no increase in extraction of PAH when acetate was infused at rates which increase tubular secretory activity. (Acetate is known to increase the efficiency of the secretory system.) This is what would be expected if cortical clearance is complete. A decrease in E_{PAH} was found following the infusion of both acetylcholine and noradrenaline. Thurau et al (151) using their photoelectric method of renal blood flow measurement, found that during the infusion of acetylcholine both cortical and medullary flows increased with the medullary flow increasing more. Also the infusion of noradrenaline reduced cortical blood flow markedly, while medullary flow was not significantly changed.

These changes in flow distribution are what would be expected from the reduction in the extraction ratio of PAH observed by Pitts et al (109).

From these results, the conclusion is drawn that Reubi's method may be useful for approximating the distribution of plasma flow between the renal cortex and the medulla (109).

METHODS

Methods in General

The experiments are considered in three groups:

I. Preliminary Experiments.

II. Intact Kidney Experiments in which renal function studies were made on intact dogs while breathing mixtures of CO_2 and O_2.

III. Denervated Kidney Experiments in which renal function was studied in dogs with a denervated left kidney while breathing mixtures of CO_2 and O_2.

During the experiments, the following quantities were measured:

(1) Mean arterial blood pressure (mm Hg)

(2) Mean renal venous pressure (mm Hg)

(3) CO_2 content of the inspired air (%CO_2 in O_2)

(4) P_{CO_2} of arterial blood (mm Hg)

(5) pH of arterial blood

(6) Urine flow (ml/min)

(7) I^{131}-Hippuran concentration in arterial plasma, renal venous plasma and urine (cpm)

(8) Arterial plasma and urine creatinine concentration (mg/100 ml)

(9) Hematocrit of arterial blood (% RBC)

(10) Osmolarity of arterial plasma and urine (mOsm/l)

From these measurements the following calculations were made:

(1) Extraction of Hippuran (E_{Hipp}):

$$= \frac{\text{arterial conc. of Hipp.} - \text{renal venous conc. of Hipp.}}{\text{arterial conc. of Hipp.}}$$

(2) Effective renal plasma flow: ERPF (ml/min)

$$= \frac{\text{urine conc. of Hipp. (cpm)} \times \text{urine volume (ml/min)}}{\text{arterial conc. of Hipp. (cpm)}}$$

(3) Renal plasma flow: RPF (ml/min)

$$= \frac{\text{ERPF (ml/min)}}{E_{Hipp}}$$

(4) Renal blood flow: RBF (ml/min)

$$= \frac{\text{RPF (ml/min)}}{1\text{-Hct}}$$

(5) Glomerular filtration rate: GFR (ml/min)

$$= \frac{\text{urine conc. creat. (mg\%)} \times \text{urine vol. (ml/min)}}{\text{arterial plasma conc. of creatinine (mg\%)}}$$

(6) Filtration fraction: FF

$= \text{GFR}/\text{RPF}$

(7) Osmolar clearance: C_{Osm} (ml/min)

$$= \frac{\text{osmol. conc. urine (mOsm/l) x urine vol. (ml/min)}}{\text{osmol. conc. of arterial plasma (mOsm/l)}}$$

(8) Free water clearance: C_{H_2O} (ml/min)

$= \text{urine volume (ml/min)} - C_{Osm}$ (ml/min)

(9) Renal resistance: RR (renal resistance units: RRU)

$$= \frac{\text{mean art. pres. - mean renal vein pres. (mm Hg)}}{\text{RBF (ml/min)}}$$

Group I - Preliminary Experiments

Between May 1963 and June 1965, 109 experiments were performed on mongrel dogs. During these experiments, the techniques of measuring renal blood flow were investigated in an effort to obtain an accurate method usable in our experiments. A survey of this preliminary work is given in Appendix I.

Group II - Intact Kidney Series

Thirteen experiments were performed on mongrel female dogs ranging in weight from 6.7 kg to 13.8 kg (average 9.6 kg). On these 13 dogs, 63 sets of renal function and blood flow measurements were made.

One-half hour after the subcutaneous administration of morphine, the dog was anesthetized with chloralose (60-80 mg/kg). Catheters were inserted into the femoral artery, femoral vein, left renal vein, and left and right ureters. The rate of a continuous drip of a diuretic solution consisting of 5% dextrose in 0.45% NaCl was adjusted to ensure an initial urine flow of about 0.9 to 1.0 ml/min/kidney. A priming dose of I^{131}-Hippuran, unlabelled Hippuran and creatinine was administered followed immediately by the start of a constant infusion of the same substances. A forty minute equilibration period was allowed before sampling began.

Respiratory movements were arrested with gallamine triethiodide (Flaxedil) and the animal was ventilated by positive pressure using 100% O_2. After at least 10 minutes, two consecutive 10 minute clearance periods were measured. The inspired gas mixture was then changed to either 10, 15 or 20% CO_2 in O_2. Following a 10 minute equilibration period, two consecutive 10 minute clearance measurements were made. This procedure was repeated in each experiment until a total of five different CO_2 concentrations had been breathed.

Group III - Denervated Kidney Series

This group was composed of 11 mongrel female dogs ranging in weight from 7.0 kg to 13.0 kg (average 10.2 kg). On these 11 dogs, 55 sets of renal function and blood flow measurement were made on the left denervated kidneys and 45 partial sets of measurements on the right intact kidneys. The left kidney was surgically denervated one to four weeks before the experiment. On the day of the experiment, the experimental procedure was identical to that used in Group II (Intact Series).

Methods in Detail

Many of the measurements made proved to be extremely sensitive to small procedural changes. Because the writer encountered considerable difficulty in duplicating technical procedures that were not clearly described by other workers, the methods used in this study are set forth in detail.

Anesthesia

The α-chloralose used as an anesthetic was administered intravenously as a suspension made up of 20 mg/ml in 0.6% NaCl. The anesthetic dose was found to be 65 mg/kg for α-chloralose from Nutritional Biochemical Co., while 80 mg/kg was

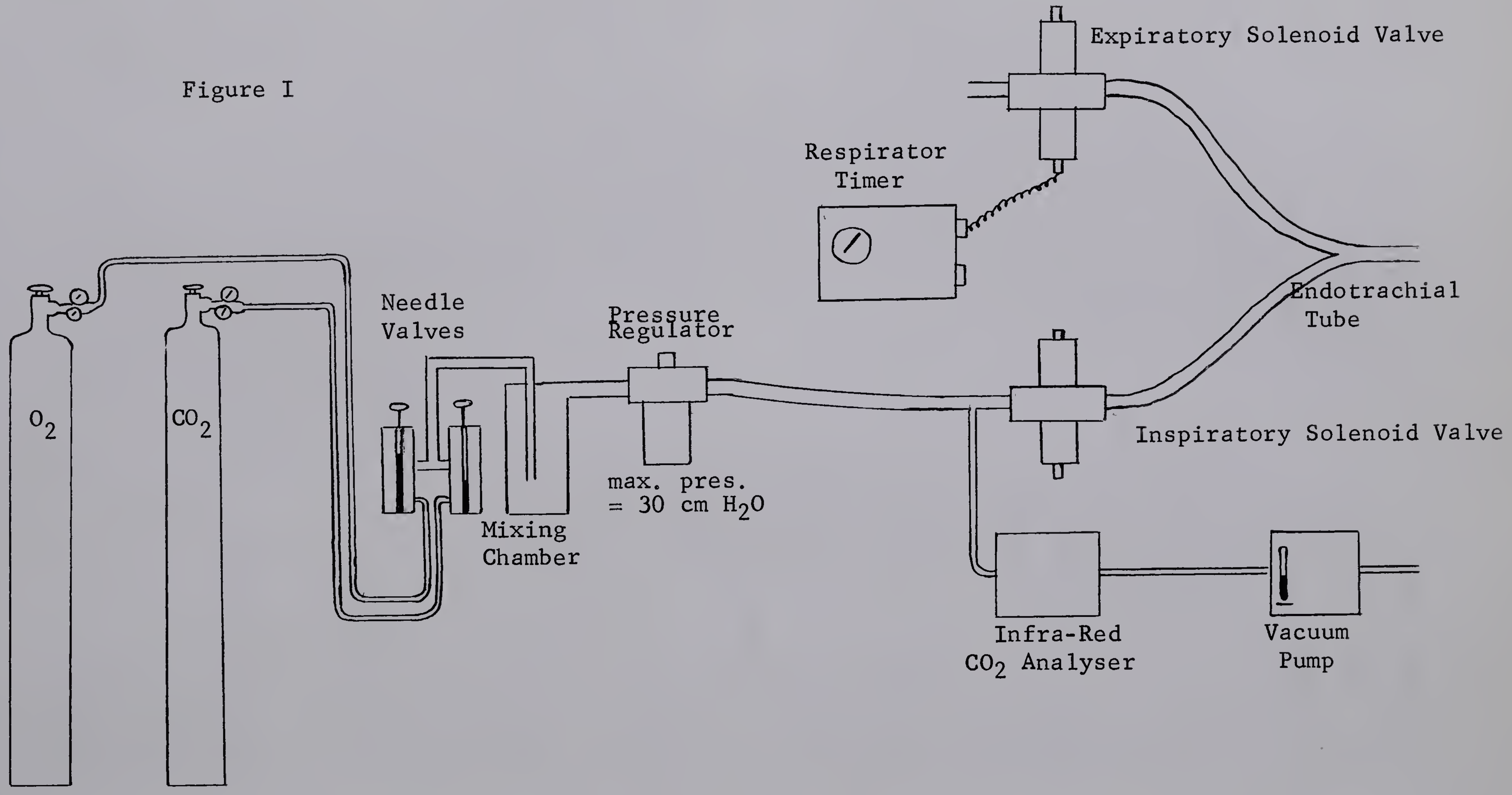
necessary for α-chloralose from Fisher Scientific Co. The α-chloralose was heated to 80°C to dissolve it in the saline solution, but was cooled to about 40°C in the syringe under cold running water before injection. Upon cooling, a fine white precipitate formed a suspension in the saline solution. Because it was necessary to administer a large volume of anesthetic (up to 40 ml for a 10 kg dog), the animal was sedated with morphine (1 mg/kg subcutaneously) one-half hour before the anesthetic was given.

Catheters

Femoral arterial and venous catheters consisting of 12 and 15 inch lengths respectively, of polyethylene tubing (PE280, ID 0.085 in, OD 0.128 in) were used. The arterial catheter was used for blood pressure measurement and for sampling of arterial blood. The venous catheter was used for the infusion and injection of various substances.

A 24 inch, #5 French, radio-opaque ureteral catheter was inserted by way of the left jugular vein into the inferior vena cava, then into the left renal vein. A small midline incision, extending about two inches above and one inch below the umbilicus, was made to aid the cannulation of the renal vein and to allow the left ovarian vein

Figure I


Expiratory Solenoid Valve
Respirator Timer
Endotrachial Tube
Needle Valves
Pressure Regulator
Inspiratory Solenoid Valve
O_2
CO_2
max. pres. = 30 cm H_2O
Mixing Chamber
Infra-Red CO_2 Analyser
Vacuum Pump

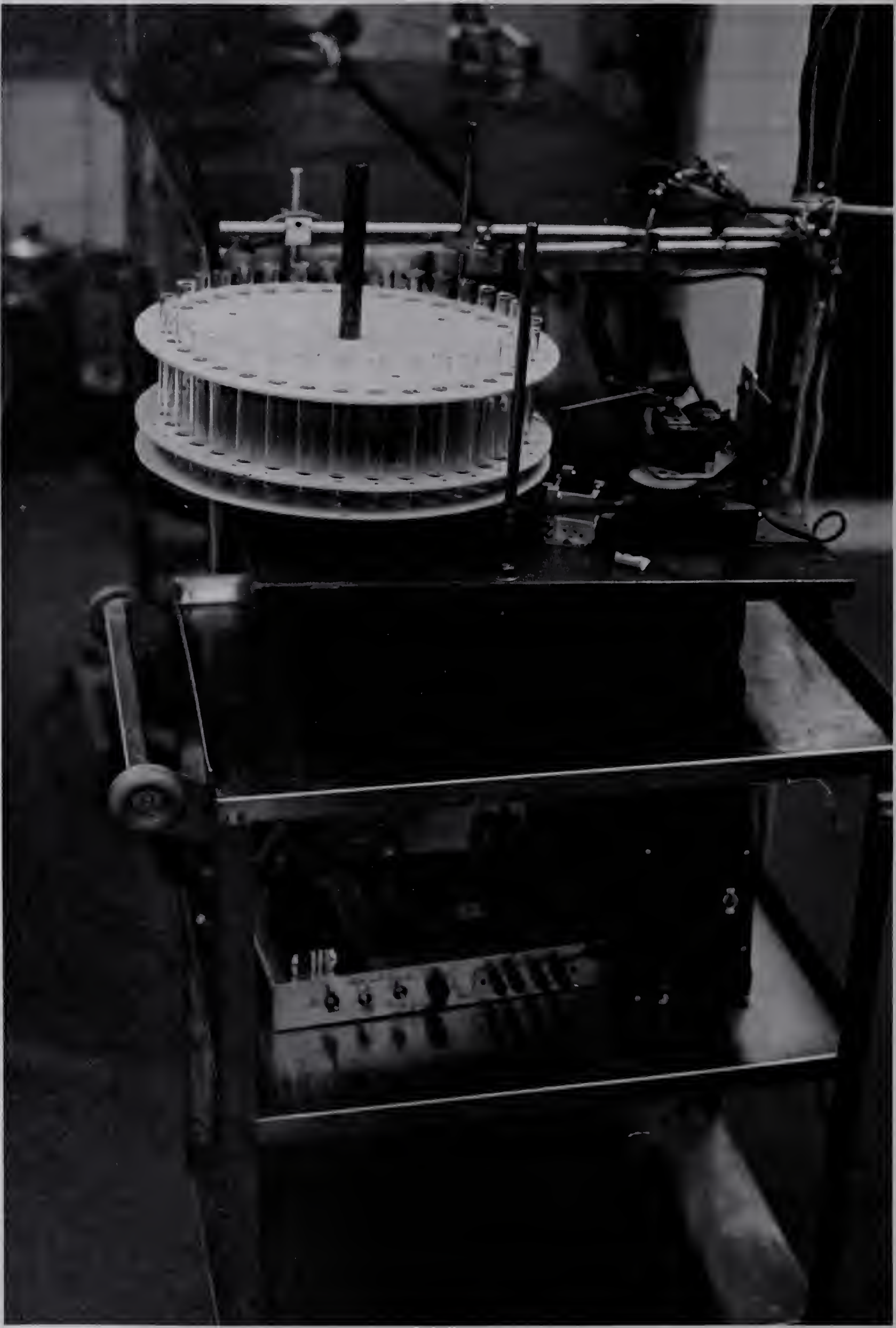
Breathing circuit showing method of administration and analysis of gas.

to be clamped with a long hemostat.

The ureters were cannulated using 18 inch lengths of polyethylene tubing (PE200, ID 0.055 in, OD 0.075 in). To insert these catheters, a midline abdominal incision extending about 2 inches cephalad from the symphysis pubis was made. The urinary bladder was drawn out and emptied if necessary with a large syringe and a 15 g needle. The two ureters were separated from surrounding tissue and cannulated approximately one inch from their point of entry into the bladder.

Control of Respiration and CO_2 Content of Inspired Air

The open circuit used for the administration and analysis of the respiratory gases is given in Figure I. The timer was set so that the solenoid valves opened alternately, giving a respiratory rate of about 30/minute. Expiration and inspiration were approximately equal in duration. This respiratory cycle was maintained constant in all experiments. CO_2 content of the inspired air was changed from 0 to 20% in O_2 by adjustment of the needle valves. The CO_2 content of inspired air was continuously analysed by an infra-red CO_2 analyser (Beckman Medical Gas Analyser, Spinco Model LB-1).



Urine Collection

Urine from the left ureter was collected in 15 ml test tubes held in a fraction collector which changed the tube every 5 minutes. The fraction collector (Figure II) was designed and built by Dr. W. H. Cottle of the Dept. of Physiology, U. of A. At the end of the experiment, the volume in each tube which had been collected over 5 minutes was measured in a 10 ml graduated cylinder.

Urine from the right ureter was collected directly into graduated 15 ml centrifuge tubes. The tube was changed manually every 5 minutes.

Blood Sampling and Analysis

Arterial and renal venous blood samples were drawn simultaneously between the 4th and 5th minute of each 10 minute clearance period.

The arterial blood sample was withdrawn from the femoral artery into a 15 ml syringe in which the dead space had been filled with heparin solution (10 mg/ml). Each arterial sample was 11-13 ml depending on the expected hematocrit. Of this sample, one ml was used for arterial hematocrit determination, one and one-half ml for pCO_2 determination and one and

---

Figure II. Fraction collector used to collect urine from the left ureter. The timer mechanism was adjusted so that the collecting tube was changed every 5 minutes.

one-half ml for pH measurement. The pCO_2 and pH of the arterial blood samples were determined electrometrically on an Epsco Blood Parameter Analyser at $37^{\circ}C$. The remaining arterial blood was centrifuged to obtain plasma for the determination of I^{131}-Hippuran, creatinine and osmolar concentrations.

The following precautions were necessary to prevent diffusion of I^{131}-Hippuran from the red cells into the plasma. Renal venous blood was drawn into ice-cold 5 ml syringes (stored at $-20^{\circ}C$) containing a small amount of heparin solution. The syringes were warmed by hand until just thawed, when a 3-4 ml sample was slowly withdrawn from the renal vein catheter over a period of 30-45 seconds. Immediately following withdrawal, the venous blood sample was transferred to an ice-cold centrifuge tube and centrifuged at 2000 rpm for 3 minutes in a cold room. After centrifugation, the tubes were returned to an ice bath and the plasma from the renal vein sample was immediately removed. It should be noted that the renal venous plasma was separated from the cells within 5 minutes of the time of withdrawal, during which the sample was either in an ice-water bath or in the centrifuge at $5^{\circ}C$.

Hematocrit

The hematocrit of arterial blood was determined in Wintrobe tubes spun in a centrifuge

for 20 minutes at 2000 rpm (R.C.F. at tip = 700 gravities).

Pressure Recording

Mean arterial and renal venous blood pressures were measured using Statham pressure transducers and a direct writing Beckman Offner Type RB Dynograph. Pressures were recorded with the dogs supine, the reference point for zero pressure being 10 cm above the table top in all experiments.

Renal Denervation

All renal denervation was performed aseptically under sodium pentobarbital anesthesia (30 mg/kg). A left flank incision was made with an electro-cautery machine (Bovie Electrosurgical Unit, Model O-3). The incision was curved below the lower rib and extended to the midline of the abdomen. The left kidney was exposed, the peritoneum separated, and all visible nerves were stripped from the renal artery, renal vein and ureter. The area was soaked with 80% ethanol several times to inactivate any remaining nerve fibres. The incision was closed with continuous sutures in the three muscle layers and interrupted sutures in the skin.

Immediately following the operation and on the following day, each dog received 400,000 I.U.

of penicillin streptomycin intramuscularly.

Clearance Materials

Creatinine, I^{131}-labelled sodium o-iodohippurate (I^{131}-Hippuran, Charles E. Frosst & Co., Montreal), and unlabelled sodium o-iodohippurate (Hippuran, Mallinckrodt Chemical Works, Montreal) were injected in a priming dose and then given as a continuous infusion calculated to maintain plasma concentrations at a constant level.

I^{131}-Hippuran dosage was calculated on the basis of total radioactivity per experiment. This dose was 7-8 μc/kg body weight. The proper volume was drawn into a syringe and 1/7 of the volume was added to the priming injection and 6/7 of the volume to the continuous infusion. These fractions correspond to the ratios of unlabelled Hippuran in the priming and sustaining infusions. The priming dose of 2.5 mg/kg unlabelled Hippuran and 50 mg/kg Creatinine, was dissolved in approximately 10 ml of distilled water. The continuous infusion contained 15 mg/kg Hippuran and 100 mg/kg Creatinine was dissolved in 50 ml distilled water. The infusion solution, with the I^{131}-Hippuran added, was infused (at a rate of 0.191 ml/min from a 50 ml syringe) using a Palmer Slow Injection Apparatus (Model F130). Both the

priming injection and the continuous infusion were administered by way of the femoral vein catheter.

Creatinine

Creatinine concentration was determined in arterial plasma and urine by a modification of the Folin-Wu alkaline picrate method (19). A tungstate protein precipitating solution was used to prepare a protein free filtrate from the arterial plasma. Two ml of plasma were diluted to 20 ml by adding 18 ml of tungstate solution (59 gm Na_2WO_4, 18.2 ml 36 N H_2SO_4 made up to 10 l with distilled H_2O). The mixture was shaken well and then allowed to stand for 15 minutes. The mixture was filtered using Whatman #2 filter paper. Three ml portions of the filtrate were then transferred to colorimeter tubes for colour development.

A 1/1000 dilution of urine was used for analysis. Three ml portions of this final dilution were transferred to colorimeter tubes for colour development.

To develop the colour, 2 ml of alkaline-picrate (0.75N HaOH:0.04M Picric acid :: 1:1, freshly mixed) was added to the 3 ml portions of protein free plasma filtrates or 1/1000 diluted urine. The colour was allowed to develop for 30 minutes and the optical density was then read at 510 mμ against a plasma

blank or distilled water blank. Optical density was determined in a Spectronic 20 - Colorimeter-Spectrophotometer (Bausch and Lomb, New York).

Solutions for plasma standards were made by dissolving creatinine in the tungstate protein precipitating solution. Standard solutions were made to contain 0.5, 1.0, 2.0, 5.0, and 10.0 mg/100 ml . Plasma standards were made as follows: 2 ml of standard solution were added to 20 ml of control plasma (plasma taken before infusion of creatinine). This was then diluted to 20 ml with tungstate protein precipitating solution and then handled in a manner identical to the plasma samples.

Urine standard solutions were made to contain 50, 100, 200, 500 and 1000 mg/100 ml distilled water. These standard solutions were diluted 1/1000 and treated the same as the urine samples.

With each set of plasma and urine samples, two standards were run, usually the 5 and 10 mg/100 ml standard for the plasma and the 100 and 500 mg/100 ml standards for the urine.

I^{131}-Hippuran Determination

0.5 ml portions of arterial plasma, renal venous plasma and urine were transferred to pyrex test tubes (15 cm x 1.6 cm OD). The tubes were placed in a well-type scintillation counter and

the gamma activity was determined. The scintillation detector (Nuclear-Chicago, Model DS5-4) was fitted with a sodium iodide, thallium activated crystal, 1-7/8 in OD, 2-1/4 in thick, with a 21/32 in well which was 1-1/2 in deep. The pulses from the scintillation detector went through a radiation analyser (Nuclear-Chicago, Model 1810) to a decade scaler (Nuclear-Chicago, Model 181A) with a resolution time of 5 μsec. The decade scaler was fitted with a timer (Nuclear-Chicago, Model T-5).

Each sample was counted for sufficient time to give a statistical accuracy of ±5%, 95% of the time. The counting time was calculated according to the following formula:

$$t = \frac{R_s+R_{b2}}{R_s{}^2V_s}$$

where t = counting time required in minutes

R_s = Bkgd. corrected cpm of sample

R_b = cpm of background

V_s = variance = 0.025 (for ±5% accuracy, 95% of the time)

The time of the beginning of each count was noted for time decay corrections.

Osmolarity Determination

Depression of the freezing point of a solution is proportional to the number of solute particles present in the solution. The solute

```
   DIMENSION A(30,3,2),B(30,3),C(30,2),D(30,3),U(30),H(30)
   DIMENSION EX(30),ERPF(30),RPF(30),RBF(30),GFR(30),FF(30)
   DIMENSION COSM(30),E(30,2)
   READ  (5     ,15     )
15 FORMAT(1X,41H                                         )
   READ  (5     ,1      ) N,BS
 1 FORMAT(1X,I2,F4.1)
   READ  (5     ,2      ) (U(I),I=1,N)
 2 FORMAT(1X,10F4.2)
   READ  (5     ,3      ) (H(I),I=1,N)
 3 FORMAT(1X,10F3.2)
   DO 20 J=1,2
20 READ  (5     ,11     ) (C(I,J),I=1,N)
11 FORMAT(1X,10F5.1)
   DO 16 J=1,2
16 READ  (5     ,13     ) (E(I,J),I=1,N)
13 FORMAT(1X,10F5.1)
   DO 7 K=1,2
   DO 7 J=1,3
 7 READ  (5     ,4      ) (A(I,J,K),I=1,N)
 4 FORMAT(1X,10F7.1)
   DO 8 J=1,3
   DO 8 I=1,N
   X1=A(I,J,1)*83.0E-09
   X2=X1*A(I,J,1)
   X3=X2+A(I,J,1)
   B(I,J)=X3-BS
   TC=A(I,J,2)*3547.0E-06
 8 D(I,J)=B(I,J)/EXP (-TC)
   WRITE (6     ,15     )
   WRITE (6     ,12     )
12 FORMAT(1X,52H EX       ERPF    RPF     RBF     GFR    FF     COSM)
   DO 10 I=1,N
   EX(I)=(D(I,1)-D(I,2))/D(I,1)
   ERPF(I)=((D(I,3)-D(I,2))*U(I))/D(I,1)
   RPF(I)=ERPF(I)/EX(I)
   RBF(I)=RPF(I)/(1.0-(0.95*H(I)))
   GFR(I)=(C(I,2)*U(I))/C(I,1)
   FF(I)=GFR(I)/RPF(I)
   COSM(I)=E(I,2)*U(I)/E(I,1)
10 WRITE (6     ,14     ) EX(I),ERPF(I),RPF(I),RBF(I),GFR(I),FF(I),COS
  1M(I)
14 FORMAT(1X,F7.4,1X,F6.2,1X,F7.2,1X,F7.2,1X,F6.2,1X,F7.4,1X,F6.2)
   END
```

Figure III – Fortran Program

concentration may be expressed as osmoles/l. One osmole corresponds to one gm molecular weight of non-ionized solute dissolved in 1000 gm H_2O.

An Aminco-Bowman Freezing Point Depression Apparatus was used to determine the freezing point depression of each sample. A standard curve was prepared with NaCl concentrations from 100 to 1850 milliosmoles/l .

One ml portions of plasma and urine were used for the determination of freezing point and from the standard curve, the osmolar concentration of each sample in milliosmoles/l (mosm/l) was obtained.

Computer Calculations

Most of the experimental results were calculated on an IBM 7040 computer using a program written in Fortran IV. A print-out of the program, sample data, and sample results are shown in Figures III, IV and V.

Samples used in the program are defined as follows:

A - data concerning I^{131}-Hippuran, either cpm or sample times (min)
C - creatinine concentrations (mg/100 ml)
E - osmolar concentrations (mosm/l)
H - hematocrits
U - urine flow (ml/min)
N - number of samples

```
PROJECT NO 750001 EXPT NO LN2 DATE 290965
100047
0130018000780048005500990093010700300064
039039048047040038040040039037
00111001080011200113001090011100112001110010800113
02620017900234004820046800386001380027600441005300
02500029200302003090030000306003070036000296002940
03120029400365004400047000343002550029600430004500
0002980000297000039700004440000450000045600004640000493000049800004940
0001150000107000017000001790000141000012000001830000157000014000001580
0172830012031001257300244790032407002594100105330016331002758700330990
0000000000000000000050000005000001000000100000015000001500000200000020
0000025000003500000400000045000005500002000000210000021500002200000225
0000225000022500002250000225000022500002250000225000022500002250000225
```

Figure IV - Data Printout

BS - background of the scintillation counter (cpm)

I - sample number (1 to N)

J - when J = 1, arterial sample

J = 2, as a subscript to A - renal venous sample

as a subscript to C or E - urine sample

J = 3, as a subscript to A - urine sample

With this subscripting system, it is easy to address any piece of data. For example A(8,2,1) refers to the cpm of the renal venous sample of sample number 8, and E(5,2) refers to the osmolar concentration of the 5th urine sample.

Referring to Fig. III, the Fortran program is arranged as follows. The first three statements labelled DIMENSION are reserving the required number of addressable locations in the memory. There then follow a series of READ statements followed by a FORMAT statement. This is instructing the computer to read a data card, where to store the information in the memory, and the position of the decimal point. For example:

```
      READ (5,2) U(I),I = 1,N
    2 FORMAT(1X,10F4.2)
```

These two statements instruct the computer to read a card (5), with the format according to statement #2 which follows. The data are urine flows, the

first one to be stored in the memory and addressed as U(1), then U(2), U(3), ...U(N). The format statement says that the first space on the card is a blank (1X). 10F4.2 states that on each card there are 10, 4-digit numbers, each having the decimal point 2 places from the right. The data on the card giving urine flows would look like line #3 of Figure IV which is as follows: 0130018000780048005500990093010700300064. This will be interpreted by the computer as 01.30, 01.80, 00.78, 00.48, 00.55, 00.99, 00.93, 01.07, 00.30, 00.64, wich will be addressed as U(1), U(2), U(3), ...U(10).

Starting with statement DO8 J = 1,3 and ending at statement 8 D(I,J) = B(I,J)/EXP(-TC) each I^{131} count is corrected for coincidence loss in the counter, background is subtracted, and each count is corrected back to zero time (the time of the first count). The next two WRITE statements print out the heading lines (Fig. V), giving project number, experiment number and date and the next line which contains the column headings for the results. The following statements, as the sample number is changed from 1 to N, contain the calculations for extraction of Hippuran (EX), effective renal plasma flow (ERPF), renal plasma flow (RPF), renal blood flow (RBF), glomerular filtration rate (GFR), filtration fraction (FF), and osmolar clearance (COSM). As

each answer is computed, it is printed out in the proper column. It may be noted that standard arithmetical statements are used for these calculations. The calculations for an experiment with 10 samples require 61 seconds of computer time.

Statistical Methods

The following statistics were calculated for all data: (i) mean ($\bar{x}$), (ii) standard deviation (SD), (iii) standard error of the mean (SEM). These were calculated according to the following formulae:

$$\bar{x} = \frac{\Sigma x}{n}$$

$$SD = \sqrt{\frac{\Sigma x^2}{n} - \left(\frac{\Sigma x}{n}\right)^2 \times \frac{n}{n-1}}$$

$$SEM = \frac{SD}{n}$$

The mean values were compared to detect significant differences. Where the difference of the means had a P value of less than 0.05, the difference was taken as being biologically significant. The significance of the difference between the means was calculated by the following formulae:

$$\delta = \sqrt{\frac{\Sigma d_1^{\,2} + \Sigma d_2^{\,2}}{(n_1-1) + (n_2-1)}} \qquad SD = \frac{\delta}{\sqrt{\frac{n_1\ n_2}{n_1 + n_2}}}$$

$$CR = \bar{x}_1 - \bar{x}_2$$

$$t = \frac{CR}{SD} \qquad df = n_1 + n_2 - 2$$

P - from a table of t values

RESULTS

In these experiments, the carbon dioxide content of the inspired air was set at four different levels: 0%, 10%, 15% and 20% CO_2 in O_2. Because of variations in ventilation, the Pa_{CO_2} attained varied somewhat. The Pa_{CO_2} values reached during the clearance measurements were grouped into four ranges, and the experimental results are plotted on the graphs at the mean Pa_{CO_2} within each range. These ranges are shown in Table 1.

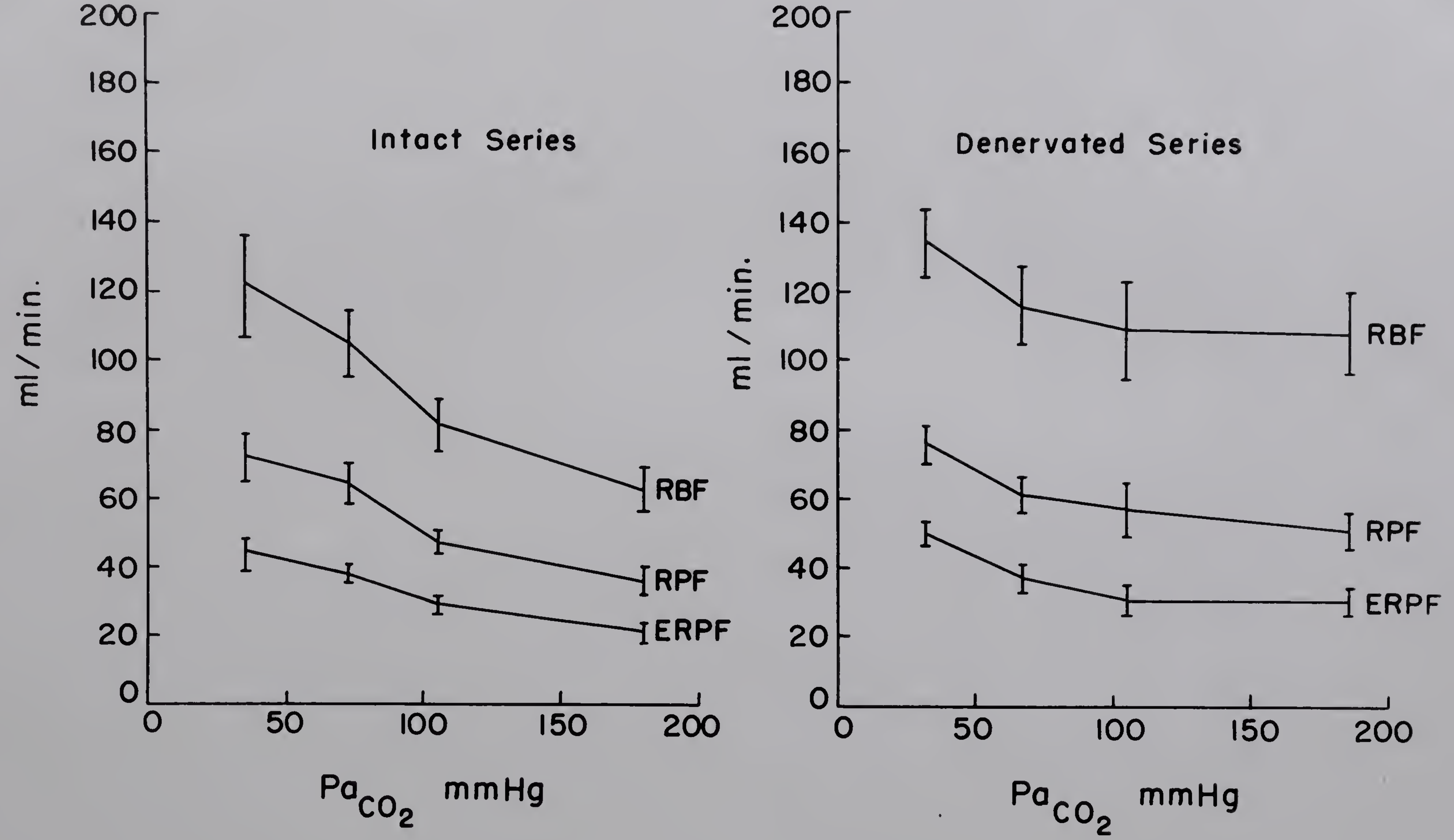
The mean values of Pa_{CO_2} differ by less than the standard error in the two series of experiments. For descriptive purposes, the Pa_{CO_2} ranges for which results will be listed are referred to as: Range 1 - 35 mm Hg, Range 2 - 70 mm Hg, Range 3 - 112 mm Hg and Range 4 - 180 mm Hg.

Table 1. Pa_{CO_2} ranges in experiments on intact and denervated kidneys.

Range #	Range in Pa_{CO_2} (mm Hg)	Intact $\bar{x} \pm$ SEM	Denervated $\bar{x} \pm$ SEM
1	25 - 45	35.2±1.43	34.9±1.22
2	46 - 90	73.5±2.45	67.6±3.07
3	91 - 130	111.9±2.75	113.4±4.49
4	131 - 210	180.1±2.60	184.3±7.16

Figure VI

Renal Plasma and Blood Flows



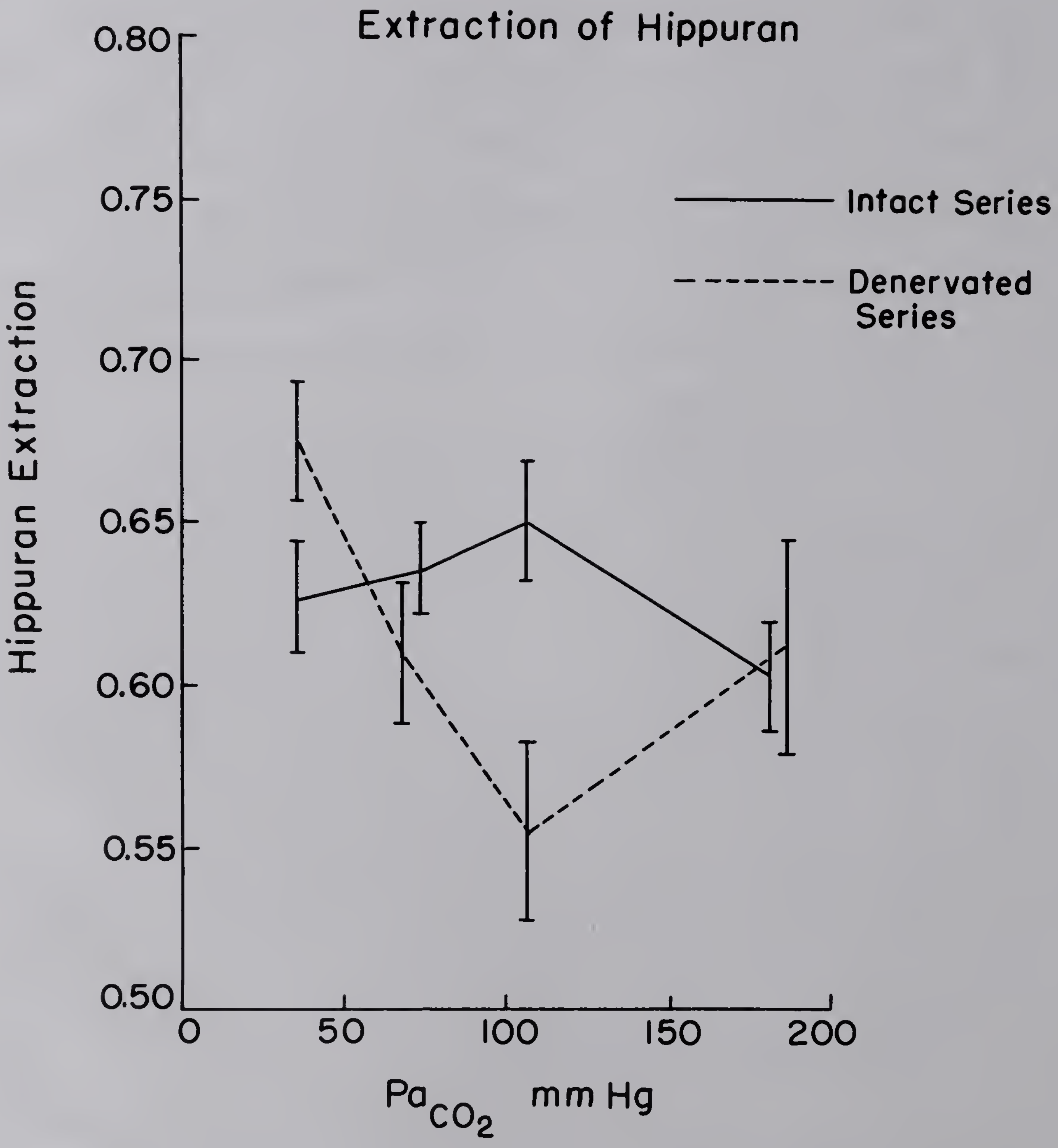
Renal Blood and Plasma Flow

Renal blood flow (RBF), renal plasma flow (RPF) and effective renal plasma flow (ERPF) showed a decrease with increasing CO_2 levels in both series. When the total decrease in RBF, RPF and ERPF was compared using paired data where available, the decrease in the intact kidneys was always significantly greater than in the denervated kidneys (RBF, -46.4% vs -21.1%, $P = < 0.025$; RPF, -51.8% vs -34.1%, $P = < 0.05$; ERPF, -61.4% vs -38.9%, $P = < 0.01$).

While the mean initial values of RBF, RPF and ERPF were all higher in the denervated kidneys, the difference was not significant. The RBF in the intact kidneys is significantly lower than in the denervated kidneys at a Pa_{CO_2} of 112 and 180 mm Hg ($P = < 0.05$ and $P = < 0.005$ respectively). The RPF and ERPF are not significantly lower in the intact kidneys compared to the denervated kidneysuntil the CO_2 tension reaches 180 mm Hg ($P = < 0.025$ and $P = < 0.025$).

Figure VI. The effect of increasing CO_2 tension on Renal Plasma and Blood Flows in intact and denervated kidneys. The vertical bars = ±1 SEM.

Figure VII


Extraction of Hippuran
Intact Series
Denervated Series
Hippuran Extraction
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0
50
100
150
200
Pa_{CO_2} mm Hg

Extraction of Hippuran

In the intact kidneys, the extraction of Hippuran decreases between CO_2 tensions of 112 and 180 mm Hg ($P = < 0.005$). At lower CO_2 tensions (35 to 112 mm Hg) there appears to be little change.

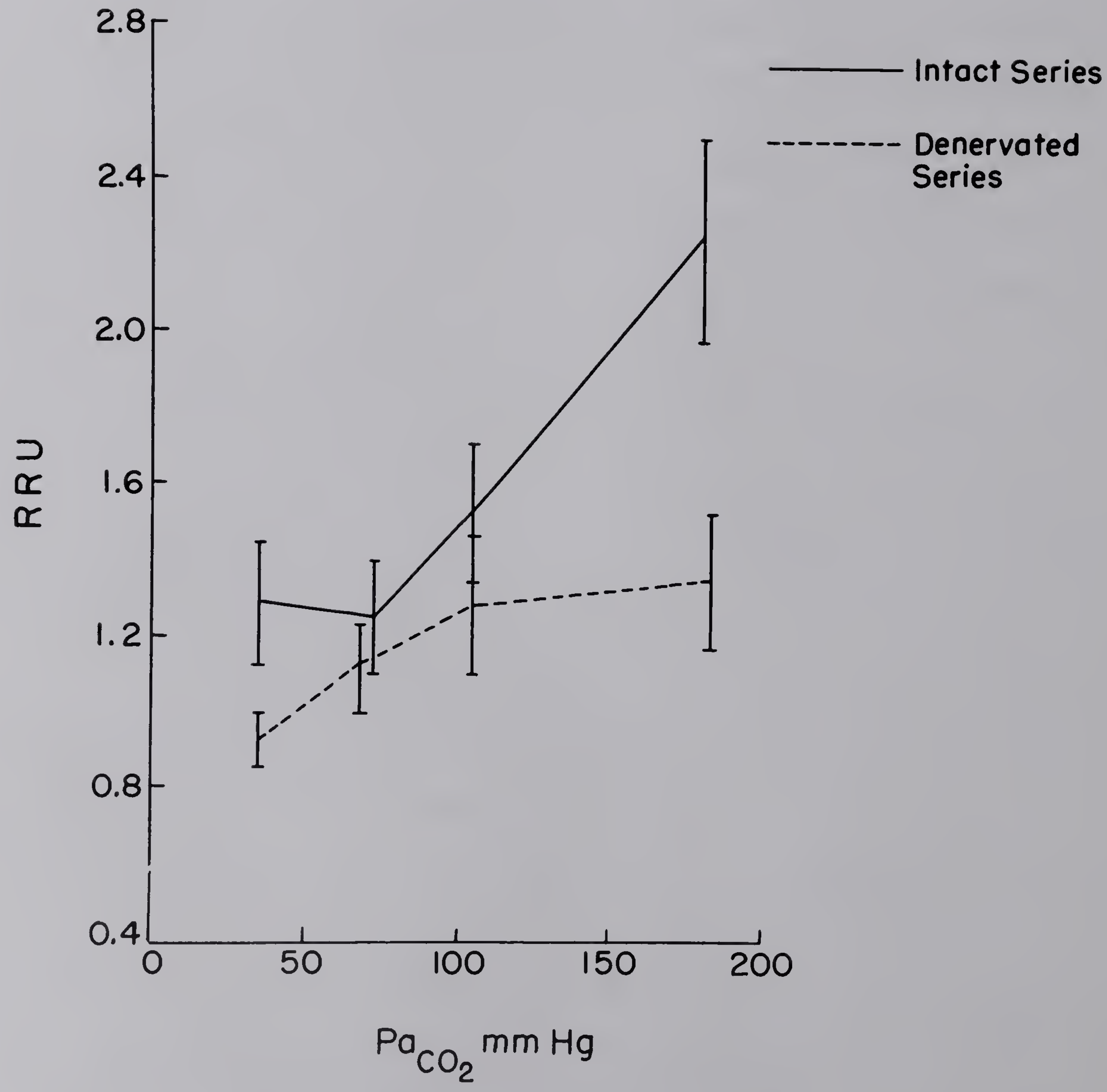
Hippuran extraction in the denervated kidneys showed a precipitous decrease at low CO_2 tensions between 35 and 112 mm Hg ($P = < 0.005$). At higher levels of CO_2, between 112 and 180 mm Hg, while extraction seems to increase again, this increase is not significant ($P = < 0.10$).

The initial value of extraction in the denervated kidneys (at $Pa_{CO_2} = 35$ mm Hg) is significantly higher than it is in the intact kidneys ($P = < 0.025$). At a $PaCO_2$ of 112 mm Hg, the extraction in the denervated kidneys has become significantly lower than in the intact kidneys ($P = < 0.005$). At the highest CO_2 levels (180 mm Hg) the extraction of Hippuran in the two series is almost identical.

Figure VII. Effect of increasing CO_2 tension on the Extraction of Hippuran in intact and denervated kidneys. Vertical bars represent ± 1 SEM.

Figure VIII

Renal Resistance



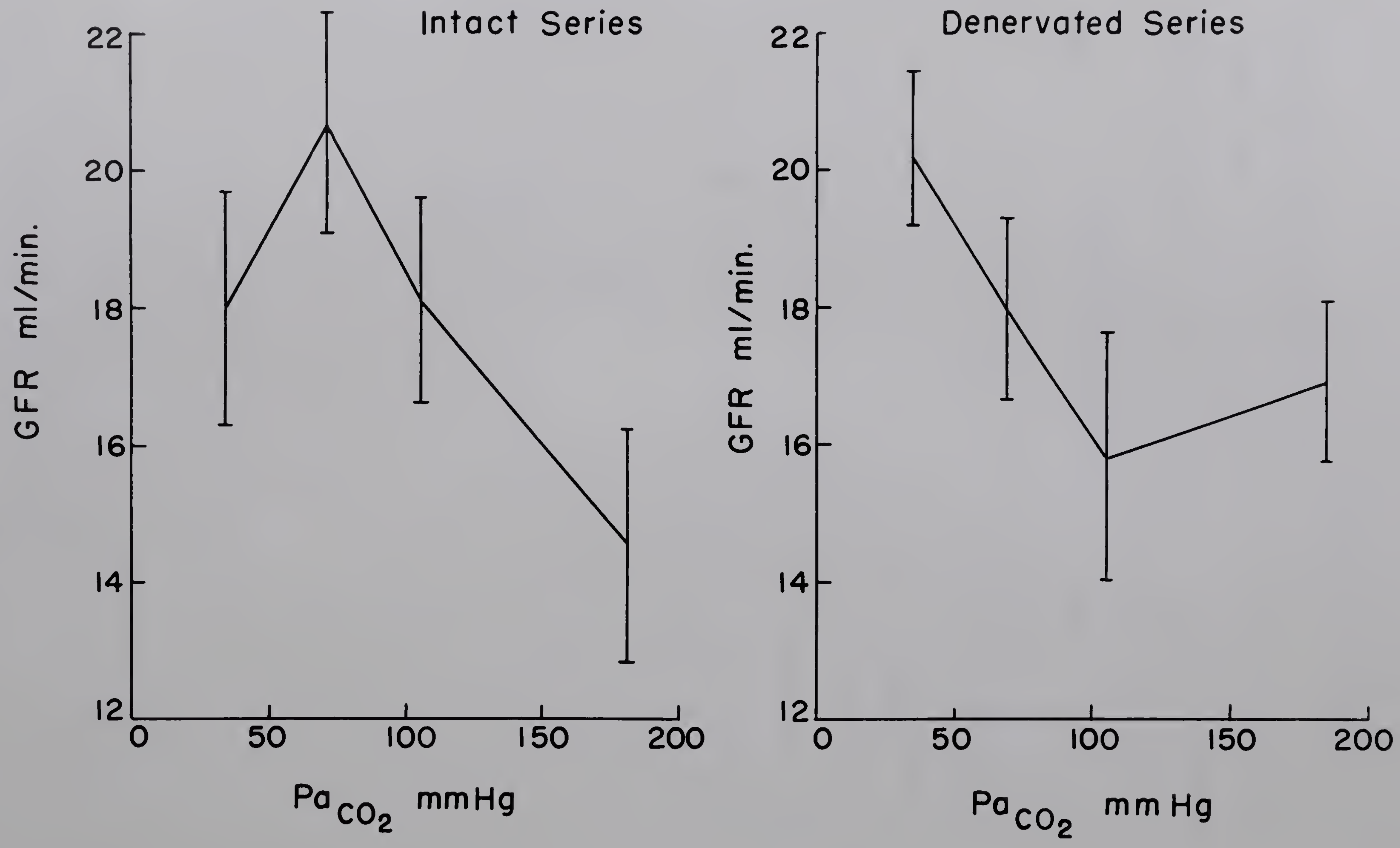
Renal Resistance

Both intact and denervated kidneys show a significant increase in renal resistance (RR) as Pa_{CO_2} is increased from 35 to 180 mm Hg (P values of 0.005 in each series), but the increase in RR in the intact kidneys (Δ = +141%) is significantly greater than that in the denervated kidneys (Δ = +35%, $P = < 0.025$). The initial value of RR in the intact kidneys is significantly higher than is the initial value in the denervated kidneys ($P = < 0.025$). However, at a Pa_{CO_2} of 70 mm Hg, the RR is very similar in the two series.

Figure VIII. The effect of increasing CO_2 tension on the Renal Resistance of intact and denervated kidneys. The vertical bars are ± 1 SEM.

Figure IX

Glomerular Filtration Rate



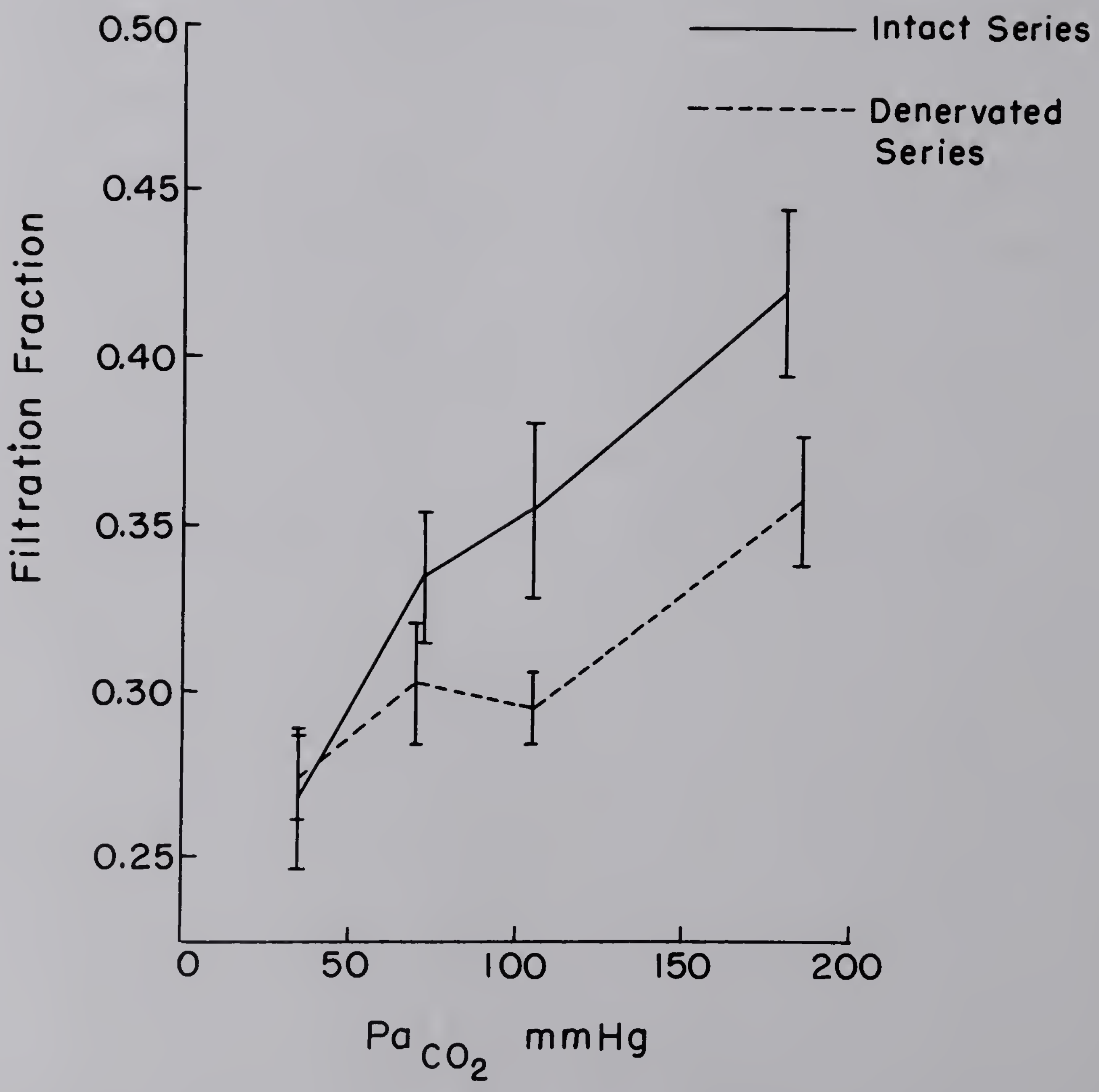
Glomerular Filtration Rate

The glomerular filtration rate (GFR) decreased significantly in both the intact ($P = 0.05$) and the denervated ($P = 0.025$) kidneys with increasing levels of CO_2 from 35 to 180 mm Hg. The decrease in the denervated kidneys occurred over the Pa_{CO_2} range 35 to 112 mm Hg so that the GFR was significantly lower than the initial value at a Pa_{CO_2} of 112 mm Hg ($P = < 0.025$). In the intact kidneys this decrease did not occur until the Pa_{CO_2} was greater than 70 mm Hg. An increase in mean GFR between 35 and 70 mm Hg in the intact kidneys was not significant but the decrease in GFR in the intact kidneys which occurred between CO_2 tensions of 70 and 180 mm Hg was highly significant ($P = < 0.005$).

Figure IX. The effect of increasing CO_2 tension on the Glomerular Filtration Rate in intact and denervated kidneys. The vertical bars = ± 1 SEM.

Figure X

Filtration Fraction



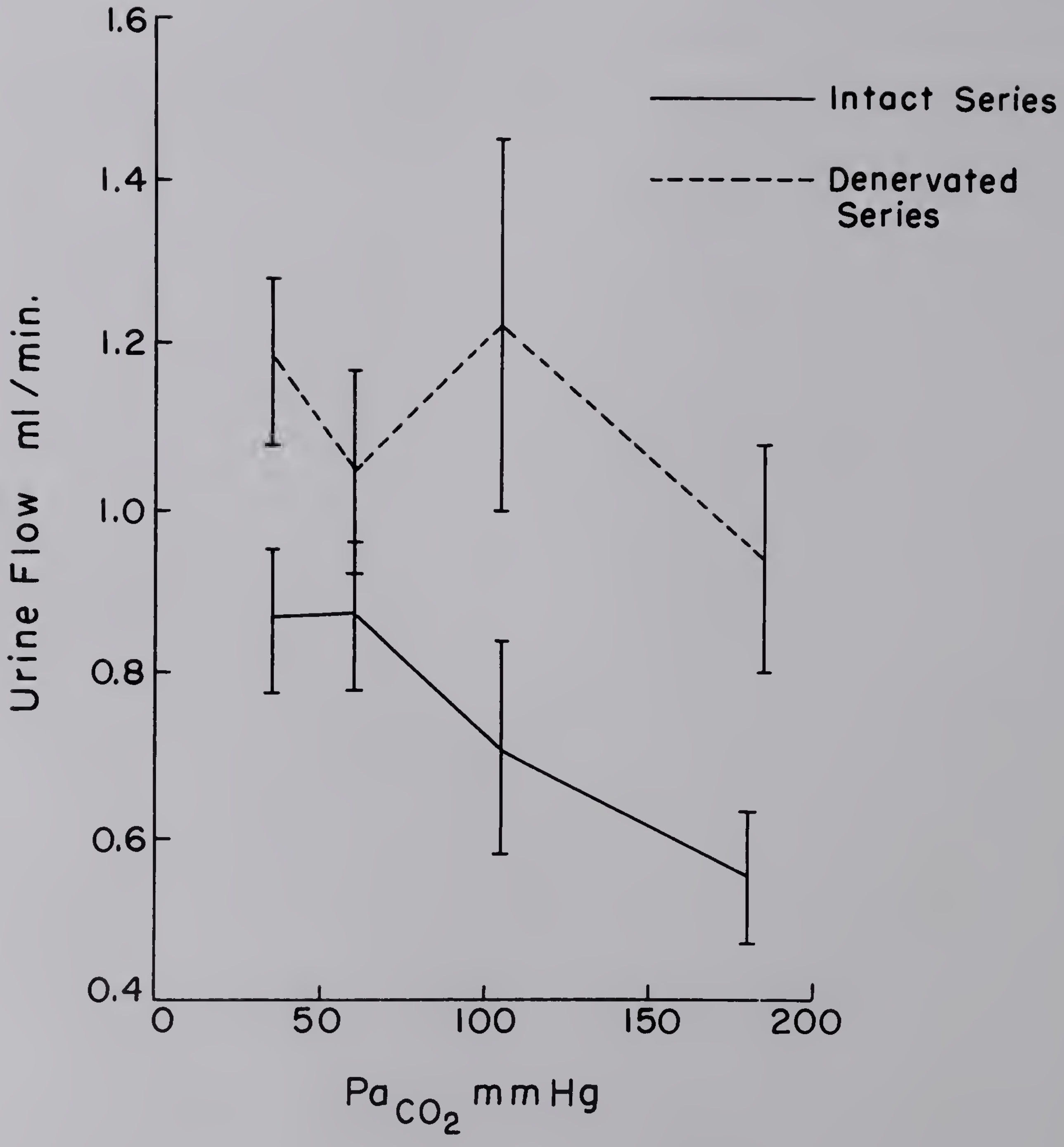
Filtration Fraction

The filtration fraction (FF) increased significantly in both the intact and the denervated kidneys with increasing CO_2 tension. The initial values in the two series were almost identical at a CO_2 tension of 35 mm Hg, but at a CO_2 tension of 180 mm Hg, the FF in the intact kidneys was significantly greater than the FF in the denervated kidneys ($P = < 0.025$).

Figure X. The effect of increasing CO_2 tension on the Filtration Fraction in intact and denervated kidneys. The vertical bars =±1 SEM.

Figure XI

Urine Flow



Urine Flow

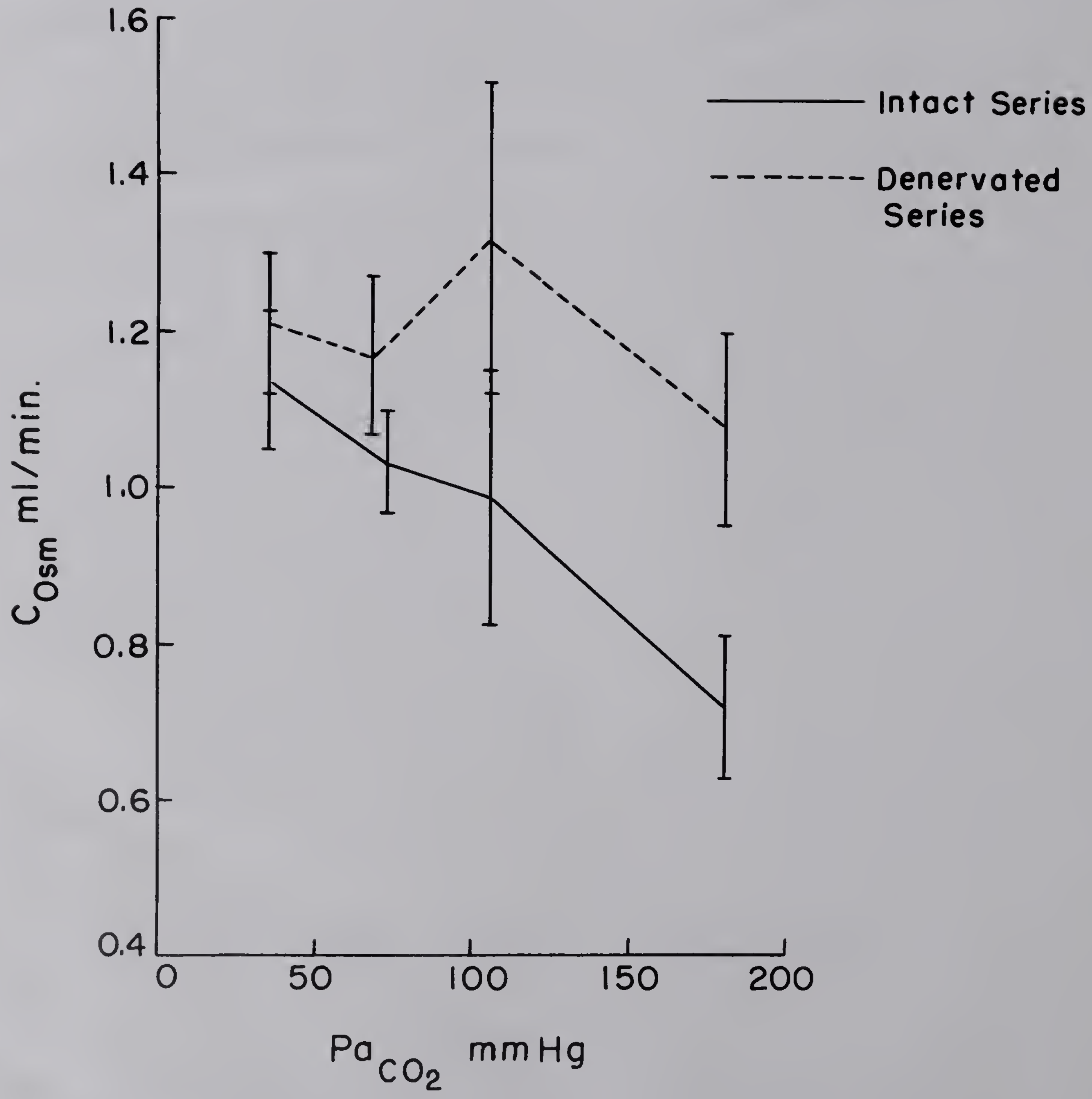
The urine flow in both the intact and the denervated kidneys appears to decrease with increasing levels of CO_2, but only the urine flow in the intact kidneys shows a significant decrease from a Pa_{CO_2} of 35 to 180 mm Hg ($P < 0.025$).

The urine flow in the denervated kidneys is generally higher than the urine flow in the intact kidneys, the difference being significant at CO_2 tensions of 35, 112 and 180 mm Hg (P values of <0.025, <0.025, and <0.01 respectively). Any decrease which does occur in either series does not do so until Pa_{CO_2} is elevated considerably above 35 mm Hg.

Figure XI. Effect of increasing CO_2 tension on the Urine Flow in intact and denervated kidneys. The vertical bars = ± SEM.

Figure XII

Osmolar Clearance



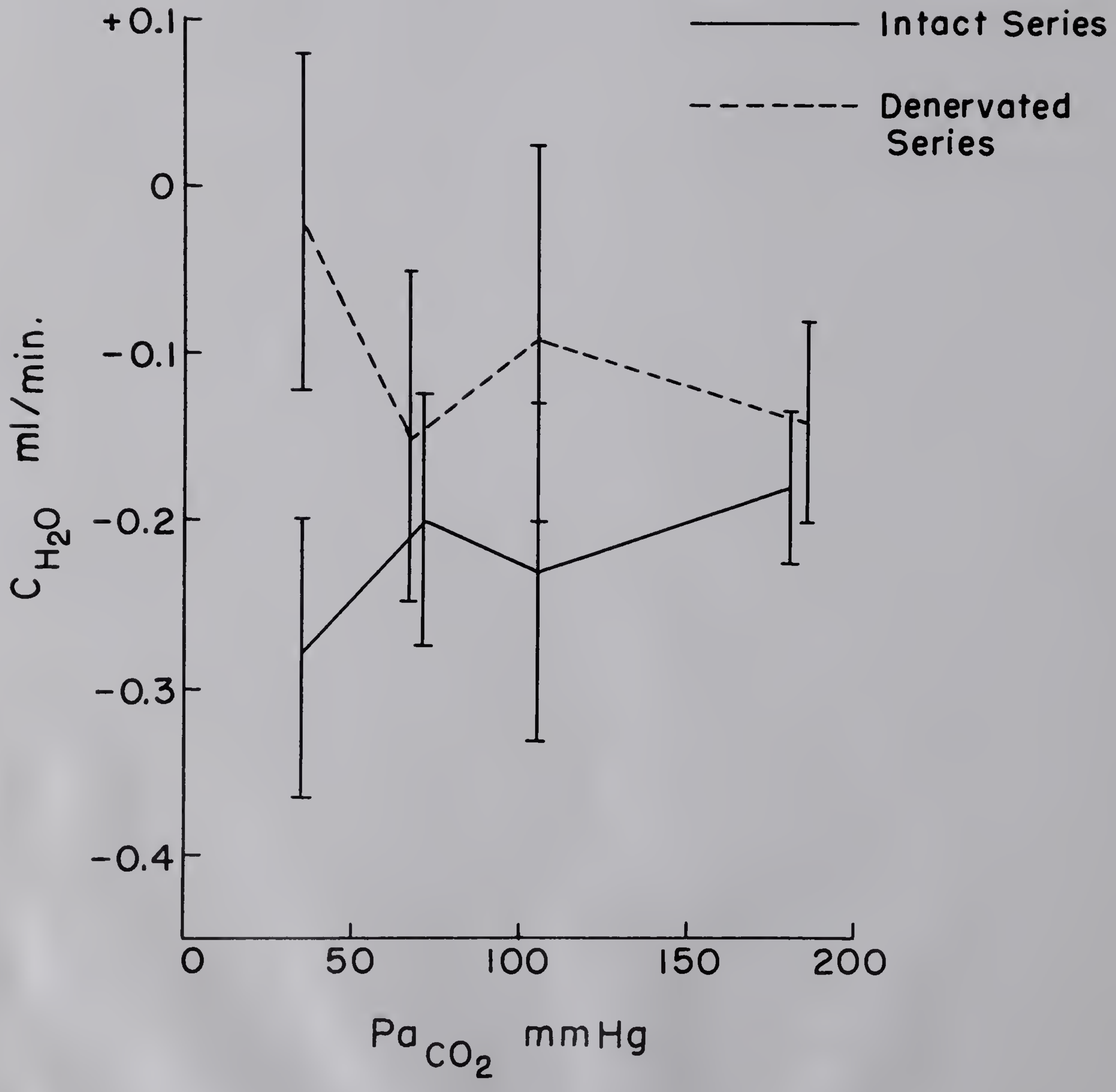
Osmolar Clearance

The osmolar clearance (C_{osm}) appears to decrease with increasing levels of CO_2 in both the intact and the denervated kidneys. However the decrease (Pa_{CO_2} 35 - 180 mm Hg) is significant only in the intact kidneys ($P < 0.005$). When a study is made of paired data where available, the overall change in the denervated kidneys ($\Delta = \pm 10\%$) is significantly different from the total change in the intact kidneys ($\Delta = -26\%$) with a P value of 0.05.

Figure XII. The effect of increasing CO_2 tension on the Osmolar Clearance in intact and denervated kidneys. The vertical bars = ±1 SEM.

Figure XIII

Free Water Clearance

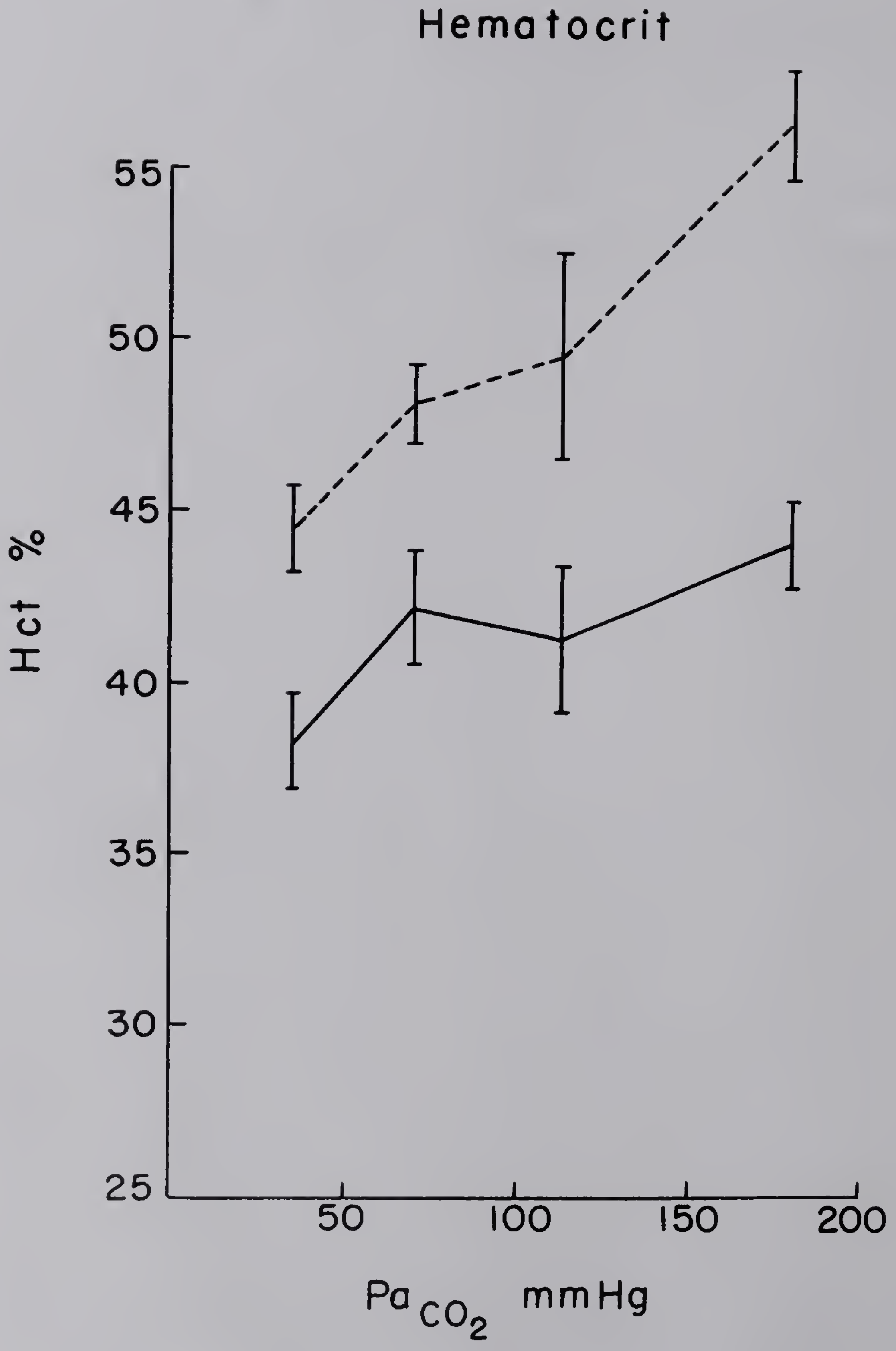


Free Water Clearance

The initial free water clearance (C_{H_2O}) in the denervated kidneys is significantly less negative than the C_{H_2O} in the intact kidneys ($P < 0.05$). The values of C_{H_2O} then appear to converge so that at higher levels of CO_2 tension, the free water clearances of both series are similar. There are no significant changes within either series.

Figure XIII. The effect of increasing CO_2 tension on the Free Water Clearance of intact and denervated kidneys. The vertical bars =±1 SEM.

Figure XIV


Hematocrit
Intact Series
Denervated Series
Hct %
55
50
45
40
35
30
25
50
100
150
200
Pa_{CO_2} mmHg

Hematocrit

The arterial hematocrits of the dogs with intact and denervated kidneys increased with increasing levels of carbon dioxide. In the dogs with intact kidneys, although the hematocrit increases at lower levels of CO_2, a significant difference from the initial values does not occur until the Pa_{CO_2} has reached 180 mm Hg ($P = < 0.025$).

The hematocrits of the animals with denervated kidneys increased more rapidly with increasing CO_2 levels so that significant increases from initial values occurred at Pa_{CO_2} values of 70, 112, and 180 mm Hg ($P = < 0.05$, < 0.025 and < 0.005 respectively).

The hematocrits of the animals with intact kidneys were significantly lower than the hematocrits of the animals with denervated kidneys at all levels of CO_2. At Pa_{CO_2} values of 35, 70, 112, and 180 the P values are 0.006, 0.006, < 0.025 and < 0.005 respectively.

Figure XIV. The effect of increasing CO_2 tension on the Hematocrit in dogs with intact and denervated kidneys. The vertical bars =±1 SEM.

DISCUSSION

The results presented in this thesis are generally consistent with most other studies relating renal blood flow to hypercapnia. There are, however, several conflicting reports. Bohr et al (18) showed a decrease in renal blood flow with apneic oxygenation which they related to increasing CO_2 level in the blood. Since they used thiopental to stop respiratory movements, the reduction in renal blood flow observed by them was probably due to central nervous system activation by the thiopental and not due to the accumulation of CO_2 by apneic oxygenation (141). However, when apneic oxygenation is induced by a muscle relaxant such as decamethonium, a reduction in renal blood flow does occur which is thought to be related to the developing hypercapnia (65, 141).

Dowds et al (41) reported that minimal renal hemodynamic changes occurred in dogs allowed

to rebreathe, even though the CO_2 content of the inspired air increased as high as 19-20%. These results are of questionable significance because when the CO_2 content of the inspired air was 19%, the arterial CO_2 content was only 51.6 vol %. Since this corresponds to a Pa_{CO_2} of only 50 mm Hg, obviously there was little or no hypercapneic stimulus. The reason for the unexpectedly low arterial CO_2 content in this work is not apparent.

The renal blood flow has been reported to either increase, not change, or to decrease when animals have been allowed to breathe gas mixtures containing increased levels of carbon dioxide. Takacs and Kallay (144), cited by Simmons and Olver (134), reported an increase in renal blood flow in rats breathing 5% and 20% CO_2 in O_2. This is the only reference to an increase in renal blood flow after breathing 20% CO_2. Emmanuel et al (45) reported that renal resistance did not change when dogs were ventilated with 20% CO_2 in oxygen for 5 min. In these experiments, renal blood flow was maintained constant by perfusion of the renal artery in situ and renal arterial pressure was measured. However, it has been noted during the preliminary work reported in the present thesis, that cannulation, prior to renal perfusion, frequently leads to abnormal hemodynamic responses. It is also

probable, that because of the short period of ventilation (5 min), Emmanuel et al (45) did not achieve a steady state.

In agreement with many authors (4, 24, 73, 83, 133, 134, 142), the results presented here show a significant decrease in renal blood flow as Pa_{CO_2} is increased. However, while it is now generally agreed that high CO_2 causes a decrease in renal blood flow, there is no agreement concerning which extra or intrarenal factors cause this decrease. It is believed by some to be caused by renal sympathetic nervous discharge (75, 142) and/or increased release of catecholamines from the adrenal medulla which is known to occur in respiratory acidosis (79, 147). But, in a recent report, Simmons et al (133) found that renal denervation did not change the renal blood flow response to breathing CO_2 in concentrations up to 30%.

The results presented in this thesis differ from those of Simmons et al (133), but are in agreement with earlier workers (75, 142), who showed that following renal denervation, the renal blood flow decrease seen in hypercapnia was not as great as that found in intact kidneys. We have shown that while initial values of blood flow (at Pa_{CO_2} of 35 and 70 mm Hg) are similar in the denervated and the intact kidneys, at Pa_{CO_2} values

above 112 mm Hg, the RBF in the intact kidneys is significantly lower than the RBF in the denervated ones.

When the renal resistance data are examined, it can be seen that in the intact kidneys the renal resistance increases sharply only above a Pa_{CO_2} of 70 mm Hg. The renal resistance changes seen here in the intact kidneys correspond closely to those reported by Simmons and Olver (134), who observed no increase and even a small decrease in renal resistance when the Pa_{CO_2} was increased from 40 to 80 mm Hg. Moreover, in their preliminary report (133), they indicate that following an initial decrease caused by a moderate increase in Pa_{CO_2}, the renal vascular resistance increased sharply at higher levels of hypercapnia. In our results, no decrease in RR was observed at lower levels of CO_2, but a sharp increase in RR did occur at high Pa_{CO_2}.

Since CO_2 caused a large increase in RR, which was significantly less in denervated kidneys, it may reasonably be assumed that the increase was mediated at least in part by the renal nerves. It is well known that an increase in Pa_{CO_2} causes an increase in sympathetic nervous system activity (18, 59, 141).

However, renal resistance in the denervated kidney did increase slightly but significantly, when Pa_{CO_2} was increased from 35 to 180 mm Hg. This increase in RR (+35%) which was much less than the increase in renal resistance observed in the intact kidneys (+141%), can be accounted for entirely on the basis of blood viscosity changes as follows: One of the responses to sympathetic nervous system discharge is contraction of the spleen, a large and active organ in the dog (13). An increase in hematocrit would therefore be expected to occur when Pa_{CO_2} is increased from 35 to 180 mm Hg. In the experiments reported here, the hematocrit changed with increasing carbon dioxide from 40-45% at a Pa_{CO_2} of 35 mm Hg, to 55-60% at a Pa_{CO_2} of 180 mm Hg. This large increase in hematocrit should result in a large increase in blood viscosity, which in turn would cause a large increase in resistance to flow. According to Poiseuille's Law, resistance varies directly with blood viscosity. From a graph presented by Bayliss (10) in which he relates relative viscosity to hematocrit, the change in blood viscosity, and hence resistance were calculated for the denervated kidneys. It was found that a resistance increase of 30% can be attributed to the viscosity change due to the observed increase in hematocrit. The overall

increase in renal resistance was 35% in the denervated kidneys, which can thus be accounted for by the change in viscosity due to the increase in hematocrit. Only 25% of the observed increase of 141% in RR in the intact kidneys can be attributed to increased viscosity. The remaining 120% resistance increase must be mediated by renal nerves since it is absent in the denervated kidneys.

It is therefore suggested that the extrarenal factors influencing RBF when carbon dioxide is increased are the increased activity of the sympathetic renal vasoconstrictor nerves, and the increase in blood viscosity, also caused by the sympathetic nervous system acting on the spleen to increase the hematocrit.

Some inferences about intrarenal hemodynamic changes could be obtained from measurements such as GFR, FF, C_{Osm} or E_{PAH} or E_{Hipp} made in conjunction with determinations of RBF. Little data that could be used for this purpose could be found in the literature.

The decrease in urine production seen here with increasing carbon dioxide (Fig. XI) is consistent with the results of other workers (2, 22, 48, 65, 141). It is the opinion of Smith and his associates (31, 135), that renal blood flow

and urine production are unrelated under normal conditions. However, Van Slyke (157) found that under abnormal conditions such as shock and dehydration, where renal blood flow is markedly reduced, a general parallelism exists between these two functions. It is probable that the oliguria seen in intact kidneys in response to hypercapnia is at least partially related to the decrease in RBF. According to Chasis et al (31) RBF appears to be controlled predominantly by the efferent glomerular arteriole. Our data are consistent with this conclusion in that the filtration fraction (FF) increased while RBF decreased with increasing carbon dioxide.

At low levels of carbon dioxide (Pa_{CO_2} of 35-70 mm Hg) a significant increase in filtration fraction was observed in the intact kidneys. In the same interval, GFR, although not changed significantly, showed a slight increase on the graph, while total renal resistance remained unchanged. This would occur if, while the efferent arterioles were beginning to constrict, the afferent arterioles dilated slightly, leaving renal resistance unchanged. The afferent arteriolar dilatation could be passive in response to the downstream efferent arteriolar constriction. Since the efferent arterioles are known to play the larger role in

the control of RBF, it seems reasonable that the renal sympathetic nerves to the efferent arterioles would have a lower threshold for discharge. Therefore, at this low level of CO_2 stimulus, one would expect that only the firing threshold of the sympathetic nerves to the efferent arterioles is exceeded.

At Pa_{CO_2} levels above 70 mm Hg, both efferent and afferent arterioles are constricted. This is indicated by the sharp increase in RR and the decrease in GFR. However, from the continuous increase in FF, it would appear that at all times, the efferent arterioles are constricted more than the afferent arterioles. At higher Pa_{CO_2} levels, the sympathetic nerves to the afferent arterioles as well as to the efferent arterioles are discharging. The efferent arteriolar nerves maintain their higher rate of discharge when compared with the nerves to the afferent arteriole.

This afferent and efferent arteriolar constriction results in a reduction in cortical blood flow. This is consistent with the results of Franklin et al (48) who observed renal cortical blanching in rabbits when exposed to high levels of carbon dioxide.

According to Reubi (114) and subsequently others (42, 80, 109, 127, 128) the extraction of PAH

varies directly with the ratio of cortical to medullary blood flow. If this is so, it can be postulated that up to a Pa_{CO_2} of 112 mm Hg (during which we observe no change in extraction), renal cortical and medullary flows are both decreased. Above a Pa_{CO_2} of 112 mm Hg, where E_{Hipp} is reduced, the cortical blood flow is decreased more than the medullary blood flow, resulting in a relative increase in medullary blood flow.

The significant decrease in C_{Osm} in the intact kidneys at Pa_{CO_2} levels above 112mm Hg is probably a reflection of the decrease in GFR which occurs at this time. While changes in C_{H_2O} are not significant, there appears to be a tendency toward the production of a more dilute urine with increasing hypercapnia. This, combined with the decrease in urine flow, also gives support to the hypothesis that at high carbon dioxide levels, the medullary blood flow is increased relative to the cortical blood flow, resulting in a decrease in the concentrating ability of the kidney. The relative increase in medullary over cortical blood flow would tend to wash out the hyperosmotic vasa recta system and thus, according to the counter-current hypothesis (56), the concentrating ability of the kidney would be impaired and a more dilute urine would be produced.

Many workers believe in the existence

of vascular shunts bypassing the cortex of the kidney (53, 54, 55, 76, 97, 127, 128, 153). It is also generally agreed that these shunts are not necessarily open in the normal intact kidney (54, 76, 127, 128). The relative increase in medullary blood flow compared to cortical blood flow seen in the intact kidneys at high Pa_{CO_2}, is probably due to a diversion of blood from the cortex to the medulla. This shunting of blood away from the cortex could be explained by two possible mechanisms. In response to the known direct dilating action of carbon dioxide on blood vessels, shunts may open which divert blood from the cortex. The other explanation could be that the cortical and juxtamedullary arterioles have a difference in reactivity to sympathetic nervous discharge and/or circulating catecholamines so that at high levels of stimulation, the cortical arterioles are constricted more than the juxtamedullary arterioles. Either or both of these mechanisms would result in a relative increase in medullary blood flow when compared to cortical blood flow.

As was shown previously, most of the change in RR in the denervated kidneys can be explained in terms of an increase in blood viscosity caused by the increase in arterial hematocrit. This calculation, which is only an approximation, assumes

that the renal vessels are constant in size and number. However, most of the change in RR takes place at low levels of CO_2 at which time, the total viscosity change is not complete, and circulating catecholamines from the adrenal medulla probably are not yet present (64, 145). At higher levels of CO_2 where one would expect to see an effect of the increased adrenaline, very little further change in renal resistance occurs. The reason for this lack of response of the resistance of the denervated kidney to the increased catecholamines is unexpected and cannot be explained from the present data.

It is noted that GFR and E_{Hipp} changed in a parallel manner in the denervated kidneys. The greatest change is seen to occur at low levels of hypercapnia (Pa_{CO_2} from 35-112 mm Hg). Since the renal nerves are not present, and, as was stated previously, the circulating catecholamines from the adrenal medulla are not increased until the Pa_{CO_2} is over 112 mm Hg, the only factors which could be responsible for these observations are a direct effect of the CO_2 on the renal blood vessels and/or an effect of the increasing viscosity of the blood.

The significant decreases in GFR and E_{Hipp} between Pa_{CO_2} levels of 35 and 112 mm Hg in the denervated kidneys are indicative of a reduction in cortical blood flow. No significant change occurs in FF in this range of hypercapnia so probably the

efferent and afferent arterioles are being affected to the same degree. While total RPF also decreases in this range, it is necessary to postulate a redistribution of blood flow with cortical flow being reduced more than medullary flow to explain the large decrease in E_{Hipp}. In the presence of the dilating effect of CO_2 on blood vessels and in the absence of any renal vasoconstrictor discharge in the denervated kidneys, it is possible that the shunts open at a lower CO_2 level than in the intact kidneys.

It is interesting to note that the dogs with denervated kidneys had a significantly higher hematocrit at all levels of CO_2 when compared with the dogs with intact kidneys. The reason for this higher hematocrit is definitely established. The blood loss at all denervation operations was minimal, so it is unlikely that hematopoiesis would have been stimulated by the operation. From a discussion with the director of the Vivarium, Dr. D. Secord, the most likely explanation of the increased hematocrits in the animals with denervated kidneys is that these animals were in much better physical condition than the intact animals. The intact animals were used within 3 or 4 days of their arrival in the Vivarium, while the animals with denervated kidneys had from 2 to 5 weeks of good care and feeding. Similar

changes in hematocrit attributable to this reason, have been observed previously by other members of the department (personal communication). Since all the denervated dogs had a higher hematocrit, an increase in hematocrit caused by the hypercapnia would cause a relatively larger increase in blood viscosity. This is because the curve relating blood viscosity to hematocrit is exponential in form (10) so that an increase in hematocrit from 50 to 55% causes a much greater increase in blood viscosity than an increase in hematocrit from 40 to 45%. Therefore because of this higher viscosity in the denervated kidneys, viscosity will probably be much more important in the control of intrarenal hemodynamics in the denervated kidneys than in the intact kidneys.

Pappenheimer and Kinter (106) on the basis of cell separation, which has since been confirmed by other workers (32, 77, 82, 155), believe that the cell separation occurs primarily in the interlobular arteries. The hematocrit therefore becomes progressively higher toward the outer cortex. According to Chinard et al (32), an increased arterial hematocrit increases the phenomenon of plasma skimming so the accumulation of red cells in the outer cortex would be increased both by the increase in arterial hematocrit occurring with the hypercapnia, and by the increased cell separation caused by the increased hematocrit.

Cortical glomeruli are usually considered to have a higher resistance to blood flow than the juxtamedullary glomeruli which have wide efferent arterioles leading into the vasa recta system. Therefore, cortical blood flow could be expected to decrease because of the combined high glomerular resistance and the greatly increased cortical hematocrit, resulting in an increased viscosity. According to Burton (28), at hematocrits over 60%, considerable deformation of red cells occurs and there may even be a tendency toward sludging of blood in cortical glomeruli as reported by Warner (161). The overall increase in renal resistance which occurs in the denervated kidney may therefore be due to a combination of the effects of blood viscosity, especially in the cortex, and the opening of some shunts which bypass the cortex.

The increase in FF at Pa_{CO_2} levels above 112 mm Hg results from a slight increase in GFR and a slight decrease in RPF. Although the increase in FF is significant, the changes in GFR and RPF are not. The increase in FF must be due to a relative increase in efferent to afferent arteriolar resistance. One possible explanation of the increase in FF at Pa_{CO_2} levels above 112 mm Hg is that the increased circulating catecholamines, which are known to be present at this level of CO_2 (145),

are constricting the efferent arterioles more than the afferent arterioles. Since the renal blood flow is controlled mostly by changes in the efferent arteriole (31) it may be that the efferent arteriole is more sensitive to the catecholamines than the afferent arteriole. Circulating catecholamines also are thought to have a greater effect on downstream renal venous segments (166) so with the increasing downstream resistance due to efferent arteriolar and perhaps venous segment constriction, the afferent arterioles may be passively dilating so that no increase in total renal resistance occurs.

C_{Osm}, C_{H_2O}, and urine flow follow parallel courses in the denervated kidneys but the observed changes are not significant. The fact that urine flow is maintained when GFR undergoes a significant decrease is additional evidence indicating a relative increase in medullary blood flow compared to the cortical blood flow which would impair the renal concentrating mechanism.

SUMMARY AND CONCLUSIONS

1. Acute hypercapnia was induced in anesthetized (α-chloralose) dogs with intact and denervated kidneys by ventilating them by means of positive pressure with mixtures of gas containing 10, 15 or 20% CO_2 in O_2.
2. After at least 10 minutes ventilation at any one level of CO_2, two consecutive 10 minute clearances measurements were performed.
3. During each clearance period, the following quantities were measured: C_{Hipp}, E_{Hipp}, C_{Creat}, C_{Osm}, arterial Hct, mean renal venous and arterial blood pressure, pH and P_{CO_2} of arterial blood, % CO_2 in inspired air.
4. From these measurements, the following calculations were made: ERPF, RPF, RBF, GFR, FF, RR, C_{H_2O}.
5. In the intact kidneys, at low levels of Pa_{CO_2} (35-70 mm Hg), there is evidence of efferent arteriolar constriction and afferent arteriolar

dilatation.

6. It is postulated that this low level stimulus to the sympathetic nervous system results in sympathetic discharge to only the efferent arterioles. The afferent arteriolar dilatation is probably passive, in response to the efferent arteriolar constriction.
7. At higher levels of CO_2 (Pa_{CO_2} above 70 mm Hg) the afferent arterioles also constrict, but the efferent arterioles maintain a greater constriction than the afferent arterioles.
8. At very high Pa_{CO_2} (above 112 mm Hg) there is evidence that there is a relative increase in medullary compared with cortical blood flow.
9. The relative increase in medullary blood flow could be due to opening of shunts by the direct dilating action of CO_2 on blood vessels, or to a difference in reactivity of cortical and juxtamedullary arterioles to an increase in sympathetic discharge and circulating catecholamines.
10. In the denervated kidneys, there is evidence that cortical blood flow is decreased between Pa_{CO_2} of 35 and 112 mm Hg. This decrease in cortical blood flow can be explained on the basis of shunts which open at a lower CO_2 level than in the intact kidney due to the absence of sympathetic

nervous discharge and to the direct dilating action of CO_2 on blood vessels.

11. Because the Hct is significantly higher in the dogs with denervated kidneys at all levels of CO_2 when compared to the dogs with intact kidneys, viscosity will play a much greater role in the redistribution of flow in the denervated kidneys.
12. The increased Hct causes increased plasma skimming, so Hct and thus viscosity is greatly increased in the outer cortex. Since cortical glomeruli normally have a higher resistance to flow compared with juxtamedullary glomeruli, flow will probably decrease more in the cortex than in the juxtamedullary region. This would cause cortical blood flow to decrease relative to medullary blood flow.
13. At Pa_{CO_2} levels above 112 mm Hg, when circulating catecholamines from the adrenal medulla are probably present, the efferent arterioles are constricted more than the afferent arterioles as is indicated by the increasing FF. There is a possibility that the efferent arterioles are more responsive to catecholamines when compared to the afferent arterioles.

BIBLIOGRAPHY

1. Abrams, H. L., E. Boÿsen, K. E. Börgstrom (1962) Effects of epinephrine on the renal circulation. Radiology 79: 911-922.

2. Adolph, E. F. (1935) Effects of carbon dioxide upon urine formation and glomerular blood flow. Amer. J. Physiol. 111: 64-74.

3. Alquist, R. R., J. P. Taylor, C. W. Rawson, Jr., V. L. Sydow (1954) Comparative effects of epinephrine and levarterenol in the intact anesthetized dog. J. Pharmacol. 110: 352-360.

4. Aoki, H., H. Hilgers, E. B. Brown, Jr. (1963) Hemodynamic effects of hypercapnia. Surg. Forum 14: 232-234.

5. Aukland, K. (1962) Urine oxygen tension: lack of correlation to some renal functions. Acta physiol. scand. 55: 362-375.

6. Aukland, K., B. F. Bower, R. W. Berliner (1964) Measurement of local blood flow with hydrogen gas. Circulation Res. 14: 164-187.

7. Bacq, Z. M., F. Bremer, L. Brouha, C. Heymans (1939) Reflexes vasomoteurs chez le chat sympathetomise non anesthesie. Arch. int. Pharmacodyn. 62: 460-473.

8. Barbour, A., G. W. Bull, B. M. Evans, N. C. Hughes Jones, J. Logothatopoulos (1953) The effect of breathing 5-7% CO_2 on urine flow and mineral excretion. Clin. Sci. 12: 1.

9. Baxter, C., T. Shires (1961) Successful perfusion of the canine kidney in vivo. Surg. Forum 12: 132-133.

10. Bayliss, L. E. (1960) in Flow Properties of Blood and Other Biological Systems. A. L. Copley, G. Stainsby, ed. Pergamon Press, New York, p. 39.

11. Behnke, A. R. (1941) Certain physiological principles underlying resuscitation and oxygen therapy. Anesthesiology 2: 245-260.

12. Bert, P. (1878) La pression barométrique (Recherches de physiologie expérimentale) G. Masson, Paris, p. 610.

13. Billings, H. H., E. B. Brown, Jr. (1955) Effect of splenectomy on changes in plasma and blood volume produced by inhalation of 30% and 40% CO_2 in dogs. Amer. J. Physiol. 180: 363-366.

14. Blake, W. D. (1961) Intrarenal distribution of flow as influenced by noradrenaline. Fed. Proc. 20: 407.

15. Block, J. B., B. A. Burrows (1958) Renal clearance of I^{131}-Diodrast at low plasma concentration. Clin. Res. 6: 284.

16. Bohr, D. F., D. C. Brodie, D. H. Chen (1958) Effect of electrolytes on arterial muscle contraction. Circulation 17: 746-749.

17. Bohr, V. C., R. H. Helmendach (1954) Effect of diffusion respiration on duration of vagal cardiac arrest. Proc. Soc. exp. Biol. N.Y. 85: 54-56.

18. Bohr, V. C., R. J. Ralls, R. E. Westerheyer (1958) Changes in renal function during induced apnea of diffusion respiration. Amer. J. Physiol. 194: 143-148.

19. Bousnes, R. W., H. H. Taussky (1945) On the colorimetric determination of creatinine by the Jaffé reaction. J. biol. Chem. 158: 581-591

20. Bradford, J. R. (1889) The innervation of the renal blood vessels. J. Physiol. 10: 358-407.

21. Bradley, S. E., R. J. Bing (1942) Renal function in the harbor seal (Phoca vitulina L.) during asphyxial ischemia and pyrogenic hyperemia. J. cell. comp. Physiol. 19: 229-237.

22. Brassfield, C. R., V. G. Behrmann (1941) A correlation of the pH of arterial blood and urine as affected by changes in pulmonary ventilation. Amer. J. Physiol. 132: 272-280.

23. Brody, M. J., H. W. Fischer (1964) Angiographic analysis of renal vasoconstriction. Amer. J. Physiol. 207: 495-499.

24. Brooker, W. J., J. S. Ansell, E. B. Brown, Jr. (1960) Effects of respiratory acidosis on renal blood flow. Surg. Forum 10: 869-872.

25. Brown, E. B., Jr. (1957) Plasma electrolyte composition in dogs breathing high CO_2 mixtures. School of Aviation Medicine, USAF, Report No. 57-115.

26. Brun, C., C. Crone, H. G. Davidsen, J. Fabricius, A. T. Hansen, N. A. Lassen, O. Munc (1955) Renal blood flow in anuric human subjects determined by use of radioactive Krypton^{85}. Proc. Soc. exp. Biol. N.Y. 89: 687-690.

27. Burket, I. R. (1912) Influence of adrenaline modified by salts, on the blood pressure in the cat. Amer. J. Physiol. 30: 382-387.

28. Burton, A. C. (1965) Physiology and Biophysics of the Circulation. Year Book Medical Publishers, Inc., Chicago.

29. Bygdeman, S., U. S. von Euler (1962) The effect of respiratory acidosis upon the peripheral vascular reactivity to noradrenaline in the cat. Acta physiol. scand. 54: 138-146.

30. Cathcart, E. P., G. H. Clark (1914) Mode of action of CO_2 on blood pressure. J. Physiol. 49: 301-309.

31. Chasis, H., H. A. Ranges, W. Goldring, H. W. Smith (1938) The control of renal blood flow and glomerular filtration in normal man. J. clin. Invest. 17: 683-697.

32. Chinard, F. P., T. Enns, M. F. Nolan (1964) Arterial hematocrit and separation of cells and plasma in the dog kidney. Amer. J. Physiol. 207: 128-132.

33. Cohn, J. N., E. A. Gombos (1964) An indicator-dilution method for measuring renal blood flow in man. Clin. Res. 12: 69.

34. Conn, H. L., Jr., W. Anderson, S. Arena (1953) Gas diffusion technique for measurement of renal blood flow with special reference to the intact, anuric subject. J. appl. Physiol. 5: 683-692.

35. Corcoran, A. C., I. H. Page (1947) Renal hemodynamic effects of adrenaline and "Ispurel". Proc. Soc. exp. Biol. N.Y. 66: 148-151.

36. Currie, J. C. H., E. Ullman (1961) Polyuria during experimental modifications of breathing. J. Physiol. 155: 438-455.

37. Dale, H. H., C. L. Evans (1922) Effects on circulation of changes in carbon dioxide content of the blood. J. Physiol. 56: 125-145.

38. Deal, C. P., H. Green (1954) Effects of pH on blood flow and peripheral resistance in muscular and cutaneous vascular beds in the hind limb of the pentobarbitalized dog. Circulation Res. 2: 148-154.

39. De Maria, W. J. A., R. P. Krueger, D. M. Hawkins, A. P. Sanders, G. J. Baylin (1963) Renal artery response to epinephrine. Proc. Soc. exp. Biol. N.Y. 114: 588-591.

40. Draper, W. B., R. W. Whitehead (1944) Diffusion respiration in the dog anesthetized by sodium pentothal. Anesthesiology 5: 262-273.

41. Dowds, E. G., E. W. Brickner, E. E. Selkurt (1953) Renal response to hypercapnia. Proc. Soc. exp. Biol. N.Y. 84: 15-20.

42. Earley, L. E., R. M. Friedler (1965) Changes in renal blood flow and possibly intrarenal distribution of blood during natriuresis accompanying saline loading in the dog. J. clin. Invest. 44: 929-941.

43. Elliot, H. C., A. A. Walker, III (1964) Fluorometric microanalysis of plasma hippuric acid and comparison of hippurate with p-aminohippurate clearance in dogs. Proc. Soc. exp. Biol. N.Y. 116: 268-270.

44. Elpers, M. J., E. E. Selkurt (1962) Effects of albumin infusion on renal function in the dog. Amer. J. Physiol. 205: 153-161.

45. Emanuel, D. A., M. Fleishman, F. J. Haddy (1957) Effect of pH change upon renal vascular resistance and urine flow. Circ. Res. 5:607-611.

46. Fenn, W. O., T. Asamo (1956) Effects of carbon dioxide inhalation on potassium liberation from the liver. Amer. J. Physiol. 185: 567-576.

47. Forester, R. P., J. Nyboer (1955) Effect of induced apnea on cardiovascular renal function in the rabbit. Amer. J. Physiol. 183: 149-154.

48. Franklin, K. J., L. E. McGee, E. A. Ullmann (1951) Effects of severe asphyxia on the kidney and urine flow. J. Physiol. 112: 43-53.

49. Furchgott, R. F., S. Bhadrokom (1953) Reactions of strips of rabbit aorta to epinephrine, isopropylarteronal, sodium nitrate and other drugs. J. Pharmacol. 108: 129-143.

50. Gellhorn, E. (1943) Autonomic Regulation. Interscience Publications Inc., New York.

51. Goldberg, M. (1962) Studies of the acute renal effects of hemolyzed red blood cells in dogs including estimations of renal blood flow with Krypton^{85}. J. clin. Invest. 41: 2112-2122.

52. Goldstein, J. D., E. L. DuBois (1927) Effect on circulation in man of rebreathing different concentrations of carbon dioxide. Amer. J. Physiol. 81: 650-659.

53. Gömöri, P., K. Kállay, Z, Nagy, Z, Szabó (1964) The problem of the arterio-venous anastomoses of the kidney. II. Effect of human serum albumin and dihydralazine on the opening of renal shunts. Acta med. (Hung.) 20: 159-168.

54. Gömöri, P, Z. Nagy, I. Jakob (1964) The problem of the arterio-venous anastomoses in the kidney. I. Renal extraction of PAH and O_2 in dehydration, haemorrhagic hypertension and arterial hypoxia. Acta med. (Hung.) 20: 153-158.

55. Gömöri, P., B. Zolnai, Z. Nagy, I. Jakob, Á. Mészáros (1964) The problem of renal ischemia and of the arterio-venous anastomoses of the kidney. III. New corrosion studies in dehydration, haemorrhagic, traumatic and ischaemic shock, arterial hypoxia and after serum albumin treatment. Acta med. (Hung.) 20: 169-183.

56. Gottschalk, C. W. (1964) Osmotic concentration and dilution of the urine. Amer. J. Med. 36: 670-685.

57. Greenlaw, R. H., W. D. Hudgins (1964) Correlation of renal clearance techniques in dogs. Para-aminohippuric acid compared with radio-iodinated Hippuran. J. Nucl. Med. 5: 453-461.

58. Gremels, H., E. H. Starling (1926) On influence of hydrogen ion concentration and of anoxemia upon the heart volume. J. Physiol. 61: 297-304.

59. Gruber, C. M., C. M. Gruber, Jr., K. S. Lee (1952) A study of the effect of the thiobarbiturates on the cardio-vascular system. Arch. int. Pharmacodyn. 91: 461-488.

60. Habif, D. V., E. M. Papper, H. F. Fitzpatrick, P. Lawrence, D. McC. Smith, S. E. Bradley (1951) The renal and hepatic blood flow, glomerular filtration rate, and urinary output of electrolytes during cyclopropane, ether and thiopental anesthesia, operation, and the immediate postoperative period. Surgery 30: 241-255.

60(a). Henry, J. P., J. W. Pearce (1956) The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. J. Physiol. 131: 572-585.

61. Hill, Z., M. Flack (1908) The effect of excess carbon dioxide and want of oxygen upon the respiration and the circulation. J. Physiol. 37: 77-111.

62. Honig, C. R., S. M. Tenney (1957) Determinants of the circulatory response to hypoxia and hypercapnia. Amer. Heart J. 53: 687-698.

63. Houck, C. R. (1951) Alterations in renal hemodynamics in separate kidneys during stimulation of renal artery nerves in the dog. Amer. J. Physiol. 167: 523-530.

64. Houssay, B. A., E. Morinelli (1926) Adrenal secretion produced by asphyxia. Amer. J. Physiol. 76: 538-550.

65. Irwin, R. L., W. B. Draper, R. W. Whitehead (1957) Urine secretion during diffusion respiration after apnea from neuromuscular block. Anesthesiology 18: 594-602.

66. Irwin, R. L., J. E. Stone, W. B. Draper, R. W. Whitehead (1955) Urine secretion during diffusion respiration following apnea induced by intracisternal injection of procaine hydrochloride. Anesthesiology 16: 665-673.

67. Itami, S. (1912) Action of carbon dioxide on vascular system. J. Physiol. 45: 338-344.

68. Jolliffe, N., H. W. Smith (1931) The excretion of urine in the dog. II. The urea and creatinine clearance on cracker meal diet. Amer. J. Physiol. 99: 101-107.

69. Jordan, E. C., B. Meulman, G. C. Nahas (1959) The buffering of CO_2 by THAM during hypercapnia. Effects on the circulation. Fed. Proc. 18:78.

70. Kennedy, T. J. (1960) Carbon dioxide and the kidney. Anesthesiology 21: 704-716.

71. Kety, S. S., C. F. Schmidt (1948) The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure, and normal values. J. clin. Invest. 27: 476-483.

72. Kinter, W. B., J. R. Pappenheimer (1956) Renal extraction of PAH and Diodrast-I^{131} as a function of arterial red cell concentration. Amer. J. Physiol. 185: 391-398.

73. Kittle, C. F., A. Hiroshi, E. B. Brown, Jr. (1965) The role of pH and CO_2 in the distribution of blood flow. Surgery 57: 139-153.

74. Koester, H. L., J. C. Locke, H. G. Swann (1955) Effluent constrictions in the renal vascular system. Texas Rep. Biol. Med. 13: 251-271.

75. Kopecky, F. A., C. J. Rayburn, R. W. Whitehead, W. B. Draper (1952) A study of anuria occurring during apnea under diffusion respiration. Amer. J. Physiol. 168: 131-137.

76. Kovalevskii, G. V. (1963) Shunting of blood in kidneys during acute blood loss. Arkhiv Patologii 25: 24-28.

77. Kramer, K., K. Thurau, P. Deetjen (1960) Hämodynamik des Nierenmarks Capilläre Passagezeit, Blutvolumen, Durchblutung, Gewebschämatokrit und O_2 Verbrauch des Nierenmarks in situ. Pflüg. Arch. ges Physiol. 270: 251-269.

78. Krogh, A. (1941) The Comparative Physiology of Respiratory Mechanisms. University of Pennsylvania Press, Philadelphia.

79. Ligon, J. G., G. G. Nahas (1960) Comparative effects of acidosis induced by acid infusion and CO_2 accumulation. Amer. J. Physiol. 198: 1201-1206.

80. Lilienfield, L. S., W. E. Braun (1962) Redistribution of renal blood flow during mannitol administration. Clin. Res. 10: 67.

81. Lilienfield, L. S., H. C. Maganzini, M. H. Bauer (1961) Blood flow in the renal medulla. Circulation Res. 9: 614-617.

82. Lilienfield, L. S., J. C. Rose (1958) Effect of blood pressure alterations on intrarenal red cell-plasma separation. J. clin. Invest. 37: 1106-1110.

83. Little, W. M., J. W. Avera, S. W. Hoobler (1949) Effects of breathing CO_2 on blood pressure and renal circulation in normotensive and hypertensive subjects. Fed. Proc. 8: 98.

84. Long, C. N. H. (1952) Regulation of ACTH secretion. Recent Progr. Hormone Res. 7: 75-105.

85. Mackay, J. L. (1947) Effects of narcotic levels of carbon dioxide on plasma potassium and respiration in cats. Amer. J. Physiol. 151: 469-478.

86. Malvin, R. L., W. S. Wilde, Z. P. Sullivan (1958) Localization of nephron transport by stop flow analysis. Amer. J. Physiol. 194: 135-142.

87. Mathison, G. C. (1910) Action of asphyxia upon spinal animal. J. Physiol. 41: 416-449.

88. Maxwell, M. H., E. S. Breed, H. W. Smith (1950) Significance of the renal juxtaglomerular circulation in man. Amer. J. Med. 9: 216-228.

89. McDowall, R. J. S. (1931) On variation in the activity of the cardio-inhibitory centre. J. Physiol. 71: 417-430.

90. McDowall, R. J. S. (1956) The Control of the Circulation of the Blood. W. Dawson and Sons Ltd., London.

91. McGiff, J. C. (1964) The renal vascular response to hemorrhage. J. Pharmacol. 145: 181-186.

92. McNay, J. L., R. H. McDonald, Jr., L. I. Goldberg (1965) Direct renal vasodilatation produced by dopamine in the dog. Circulation Res. 16: 510-517.

93. Millar, R. A. (1960) Plasma adrenaline and noradrenaline during diffusion respiration. J. Physiol. 150: 79-90.

94. Mithoefer, J. C., H. Kazemi (1964) Effect of carbon dioxide on heart rate. J. appl. Physiol. 19: 1151-1156.

95. Möller, E., J. F. McIntosh, D. D. Van Slyke (1929) Studies of urea excretion. IV. Relationship between urine volume and the rate of urea excretion by patients with Bright's disease. J. clin. Invest. 6:427-483.

96. Moyer, J. H., C. A. Handley (1952) Norepinephrine and epinephrine effect on renal hemodynamics with particular reference to the possibility of vascular shunting and decreasing glomeruli. Circulation 5: 91-97.

97. Munkásci, I., Z. Nagy, L. Takáts, K. Kállay, P. Gömöri (1963) Vascular shunts in the renal cortex of the dog. Rev. canad. Biol. 22: 352-363.

98. Munson, P. L., F. W. Briggs (1955) Mechanism of stimulation of ACTH secretion. Recent Progr. Hormone Res. 11: 83-117.

99. Murray, J. F., I. M. Young (1963) Regional blood flow and cardiac output during acute hypoxia in the anesthetized dog. Amer. J. Physiol. 204: 963-968.

100. Nachmansohn, D. (1959) Chemical and Molecular Basis of Nerve Activity. Academic Press, Inc., New York, pp. 112-115.

101. Nahas, G. G., R. J. Reveilland, J. Strauss, I. Schwartz, M. Verosky (1963) Renal effect of tris(hydroxymethyl)-aminomethane during CO_2 load. Amer. J. Physiol. 204: 113-118.

102. Nahas, G. G., H. Rosen (1959) The buffering of CO_2 by THAM during hypercapnia. Effects on acid-base balance. Fed. Proc. 18: 111.

103. Nash, C. W., J. V. Milligan (1959) An automatic recording bubble flowmeter. Ire Trans. on Med. Electronics ME6: 274-276.

104. Nash, C. W., R. J. Shivak, E. M. Engelhardt (1965) The influence of reserpine on cardiovascular responses to noradrenaline, tyramine, and pitressin during respiratory acidosis. Canad. J. Physiol. Pharmacol. 43: 627-638.

105. Oschwadt, B. (1963) The measurement of intrarenal blood flow distribution by wash-out technique. Proc. Intern. Congress Nephrol. 2: 62-64.

106. Pappenheimer, J. R., W. B. Kinter (1956) Hematocrit ratio of blood within mammalian kidney and its significance for renal hemodynamics. Amer. J. Physiol. 185: 377-390.

107. Peters, L. (1960) Renal tubular excretion of organic bases. Pharmacol. Rev. 12: 1-35.

108. Phillips, R. A., V. P. Dole, P. B. Hamilton, K. Emerson, Jr., R. M. Archibald, D. D. Van Slyke (1945) Effects of acute hemorrhagic and traumatic shock on renal function of dogs. Amer. J. Physiol. 145: 314-336.

109. Pilkington, L. A., R. Binder, J. C. M. de Haas, R. F. Pitts (1965) Intrarenal distribution of blood flow. Amer. J. Physiol. 208: 1107-1113.

110. Portwood, R., F. Manfredi, F. Downey (1961) Renal effects of THAM buffer. Fed. Proc. 20: 416.

111. Price, H. L. (1960) Effects of carbon dioxide upon the cardiovascular system. Anesthesiology 21: 652-663.

112. Ranson, S. W., S. L. Clark (1959) The Anatomy of the Nervous System. W. B. Saunders Co., Philadelphia.

113. Renner, E., H. H. Edel, E. Buchborn (1963) Critical appraisal of the measurement of renal blood flow using inert gas mixtures. Proc. Intern. Congress Nephrol. 2: 91-93.

114. Reubi, F. (1958) Objections á la théorie de la séparation intrarénales des hématies et du plasma (Pappenheimer). Helv. med. Acta 25: 516-523.

115. Reubi, F. C., N. Gossweiler, R. Gurtler, A. Huber (1963) Methods of measuring renal circulation in man. Proc. Intern. Congress Nephrol. 2: 78-83.

116. Reubi, F. C., C. Vorburger, H. M. Keller (1962) The renal extraction of sodium para-aminohippurate (PAH) in anaemic subjects before and after a red cell transfusion. Clin. Sci. 23: 213-219.

117. Richards, A. N., O. H. Plant (1922) Urine formation in the perfused kidney. The influence of adrenaline on the volume of the perfused kidney. Amer. J. Physiol. 59: 184-190.

118. Richards, J. B., S. N. Stein (1957) Effect of CO_2 exposure and respiratory acidosis on adrenal 17-hydroxycorticosteroid secretion in anesthetized dogs. Amer. J. Physiol. 188: 1-6.

119. Richardson, J. A., E. F. Woods (1956) Effect of carbon dioxide inhalation on plasma concentrations of epinephrine and arterenol. Fed. Proc. 15: 473-474.

120. Salant, W., R. L. Johnston (1924) Response of isolated frog heart to changes in hydrogen ion concentration and adrenaline. J. Pharmacol. 23: 373-383.

121. Samaan, A., G. Stella (1935) The response of the chemical receptors of the carotid sinus to the tension of CO_2 in the arterial blood in the cat. J. Physiol. 85: 309-319.

122. Schilling, C. W., R. M. Thomson, A. R. Behnke, L. A. Shaw, A. C. Messer (1934) Studies on the effect of high oxygen pressure. Amer. J. Physiol. 107: 29-36.

123. Schneider, E. C., D. Truesdell (1922) The effects on the circulation and respiration of an increase in the carbon dioxide content of the blood in man. Amer. J. Physiol. 63: 155-175.

124. Schnermann, J., K. Thurau (1965) Mikropunktionsversuche zum Verhalten der PAH-Konzentration im Vasa recta-Blut der Goldhamsterniere. Pflüg. Arch. ges Physiol. 283: 171-181.

125. Scott, J. B., R. M. Daugherty, Jr., J. M. Dabney, F. J. Haddy (1965) Role of chemical factors in regulation of flow through kidney, hindlimb and heart. Amer. J. Physiol. 208: 813-824.

126. Selkurt, E. E. (1962) Physiology. Little, Brown and Co., Boston.

127. Selkurt, E. E. (1963) Effects of ureteral blockade on renal blood flow and urinary concentrating ability. Amer. J. Physiol. 205: 286-292.

128. Selkurt, E. E., M. J. Elpers (1963) Influence of hemorrhagic shock on renal hemodynamics and osmolar clearance in the dog. Amer. J. Physiol. 205: 147-152.

129. Shannon, J. A., H. W. Smith (1935) The excretion of inulin, xylose and urea by normal and phlorizinized man. J. clin. Invest. 14: 393-401.

130. Shannon, J. A., F. R. Winton (1940) The renal excretion of inulin and creatinine by the anesthetized dog and the pump-lung-kidney preparation. J. Physiol. 98: 97-108.

131. Sheehan, H. L. (1931) The deposition of dyes in the mammalian kidney. J. Physiol. 72: 201-246.

132. Shires, T., S. W. Eyer (1951) Studies in diffusion respiration. J. Aviat. Med. 22: 22-30.

133. Simmons, D. H., T. J. Bersentes, A. Peery (1965) The effect of acute acidosis on renal hemodynamics. Physiologist 8: 273.

134. Simmons, D. H., R. P. Olver (1965) Effects of acute acid-base changes on renal hemodynamics in anesthetized dogs. Amer. J. Physiol. 209: 1180-1186.

135. Smith, H. W. (1951) The Kidney. Structure and Function in Health and Disease. Oxford University Press, New York.

136. Smith, H. W., N. Finkelstein, L. Aliminosa, B. Crawford, M. Graber (1945) The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. clin. Invest. 24: 388-404.

137. Smith, H. W., M. H. Maxwell, D. M. Gomez, A. D. Fishman (1953) Effects of epinephrine and typhoid vaccine on segmental vascular resistance in the human kidney. J. Pharmacol. 109: 274-283.

138. Snyder, C. D., W. A. Campbell, Jr. (1920) Vascular reaction to epinephrine in perfusates of various H-ion concentration. Amer. J. Physiol. 51: 199-200.

139. Spencer, M. P., A. B. Denison, Jr., H. D. Green (1954) Direct renal vascular effects of epinephrine and norepinephrine before and after adrenergic blockade. Circulation Res. 2: 537-540.

140. Sperber, I. (1959) Secretion of organic acids in the formation of urine and bile. Pharmacol. Rev. 11: 109-134.

141. Stone, J. E., R. L. Irwin, C. D. Wood, W. B. Draper, R. W. Whitehead (1959) Renal blood flow in dogs during diffusion respiration. J. appl. Physiol. 14: 405-410.

142. Stone, J. E., J. Wells, W. B. Draper, R. W. Whitehead (1958) Changes in renal blood flow in dogs during inhalation of 30% carbon dioxide. Amer. J. Physiol. 194: 115-119.

143. Taggart, J. V. (1954) Some biochemical features of tubular transport mechanisms. in The Kidney, A Ciba Foundation Symposium, Churchill, London, pp. 65-76.

144. Takács, L. K. Kállay (1963) Effects of carbon dioxide inhalation on the circulation of the anesthetized rat. Acta physiol. (Hung.) 23: 13-19.

145. Tenney, S. M. (1956) Sympatho-adrenal stimulation by carbon dioxide and the inhibitory effects of carbonic acid on epinephrine response. Amer. J. Physiol. 187: 341-346.

146. Tenney, S. M. (1956) Mechanism of hypertension during diffusion respiration. Anesthesiology 17: 768-776.

147. Tenney, S. M. (1960) The effect of carbon dioxide on neurohumoral and endocrine mechanisms. Anesthesiology 21: 674-685.

148. Thompson, D. D., F. Kavaler, R. Zozano, R. F. Pitts (1957) Evaluation of the cell separation hypothesis of autoregulation of renal blood flow and filtration rate. Amer. J. Physiol. 191: 493-500.

149. Thorburn, G. D., H. H. Kopald, J. A. Herd, M. Hollenberg, C. C. C. O'Morchoe, A. C. Barger (1963) Intrarenal distribution of nutrient blood flow determined with Krypton^{85} in the unanesthetized dog. Circulation Res. 13: 290-307.

150. Thurau, K. (1964) Renal hemodynamics. Amer. J. Med. 36: 698-719.

151. Thurau, K., P. Deetjen, K. Kramer (1960) Hämodynamik des Nierenmarkes. II. Mitteilung. Wechselbeziehung zwischen vasculärem und tubulärem Gegenstromsystem bei arteriellen Drucksteigerungen, Wasserdiurese und osmotischer Diurese. Pflüg. Arch. ges Physiol. 270: 270-285.

152. Tobian, L., J. Martin, W. Eilers (1959) Effect of pH on norepinephrine-induced contractions of isolated arterial smooth muscle. Amer. J. Physiol. 196: 998-1002.

153. Trueta, J. A., A. E. Barclay, P. M. Daniel, K. J. Franklin, M. M. L. Prichard (1948) Studies on the Renal Circulation. 2nd Ed. Blackwell Scientific Publications, Oxford.

154. Ulbrich, K. J., K. Kramer, J. W. Boylan (1961) Present knowledge of the counter-current system in the mammalian kidney. Progr. Cardiovascular Diseases 31: 395-431.

155. Ulfendahl, H. R. (1962) Distribution of red cells and plasma in rabbit and cat kidney. Acta physiol. scand. 56: 42-60.

156. Valten, H., I. D. Wilson, S. M. Tenney (1959) CO_2 diuresis with special reference to role of the left atrial stretch receptor mechanism. J. appl. Physiol. 14: 844-848.

157. Van Slyke, D. D. (1948) The effects of shock on the kidney. Ann. intern. Med. 28: 701-722.

158. von Euler, U. S. (1959-60) Neurotransmission in the adrenergic nervous system. Harvey Lect. 55: 46-65.

159. von Euler, U.S., G. Liljestrand, Y. Zotterman (1939) Excitation mechanism of chemoreceptors of the carotid body. Skand. Arch. Physiol. 83: 132-152.

160. Vondoukis, I. J., R. J. Boucek (1962) Induced renal vasomotion in the intact dog. Amer. J. Physiol. 202: 888-892.

161. Warner, L. (1949) Some kinds of blood sludge can plug kidney glomeruli. Anat. Rec. 103: 594-595.

162. Weiner, I. M., G. H. Mudge (1964) Renal tubular mechanisms for excretion of organic acids and bases. Amer. J. Med. 36: 743-762.

163. Winton, F. R. (1931) The control of the glomerular pressure by vascular changes with the isolated mammalian kidney, demonstrated by the action of adrenaline. J. Physiol. 73: 151-162.

164. Worthen, D. M., L. N. Anderson, L. B. Hinshaw (1962) Response of isolated perfused dog kidneys to epinephrine. Amer. J. Physiol. 202: 97-102.

165. Young, W. G., Jr., W. C. Sealy, J. Harris, A. Botwin (1951) The effects of hypercapnia and hypoxia on the response of the heart to vagal stimulation. Surg. Gynec. Obstet. 93: 51-55.

166. Zimmerman, B. G., F. M. Abboud, J. W. Eckstein (1964) Effects of norepinephrine and angiotension on total and venous resistance in the kidney. Amer. J. Physiol. 206: 701-706.

TABLES

Table 2a. Extraction of Hippuran - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	0.617	0.659	0.628 0.719	0.593
I_2	0.603	0.638	0.697 0.577	0.465
I_3	0.561	0.584	0.566 0.714	0.657
I_4		0.648 0.708	0.665	0.605 0.661
I_5	0.682 0.701 0.712			0.589 0.632
I_6	0.636 0.617	0.603	0.614	
I_7	0.556 0.484	0.566		
I_8	0.775	0.681 0.760	0.689 0.712	
I_9	0.546 0.535	0.567 0.509	0.534	
I_{10}	0.652 0.726	0.665		0.650 0.622

Table 2a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{11} R	0.649 0.634	0.645		
I_{11} L	0.608 0.613	0.541		
I_{12}	0.645	0.623 0.652	0.691	0.550
I_{13}		0.624 0.708 0.685		0.586 0.625
$\bar{x}$	0.628	0.636	0.651	0.603
SD	±0.076	±0.063	±0.647	±0.054
SEM	±0.017	±0.014	±0.019	±0.016

R_1-R_3, P = $<$0.005

R_2-R_3, P = 0.07

R_1-R_2, P = $<$0.025

R_3-R_4, P = $>$0.10

Table 2b. Extraction of Hippuran - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	0.664 0.683	0.621 0.527	0.654	
D_2	0.698	0.689 0.776		0.710 0.680
D_3	0.806 0.797 0.717	0.763		0.718
D_4	0.620 0.610 0.620		0.520	0.580
D_5	0.737 0.728	0.599	0.572	0.857
D_6	0.601	0.643 0.624		0.663 0.625
D_7	0.639	0.501 0.616	0.566 0.558	
D_8	0.628	0.526 0.584	0.436	0.490

Table 2b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_9	0.585		0.575	0.445 0.539 0.564
D_{10}		0.520 0.563 0.613		0.604 0.492
$\bar{x}$	0.675	0.609	0.554	0.612
SD	±0.070	±0.083	±0.076	±0.118
SEM	±0.018	±0.022	±0.029	±0.033

R_1-R_3, $P = < 0.005$

R_2-R_3, $P = 0.07$

R_1-R_2, $P = < 0.025$

R_3-R_4, $P = > 0.10$

R_1I-R_1D, $P = 0.05$

R_2I-R_2D, $P = > 0.10$

R_3I-R_3D, $P = < 0.005$

I - Intact Kidneys

D - Denervated Kidneys

Table 3a. Renal Blood Flow - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	139.6	116.3	72.3 47.1	58.3
I_2	125.6	92.3	59.1 85.8	64.0
I_3	118.4	112.3	62.0 74.4	36.5
I_4		116.3 87.2	99.1	101.7 93.3
I_5	92.6 58.1 49.9			82.7 46.9
I_6	173.8 150.5	118.0	54.3	
I_7	286.9 255.6	236.5		
I_8	96.4	124.3 102.7	122.7 89.7	
I_9	161.3 141.1	127.1 110.9	100.5	

Table 3a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	94.7 44.1	61.4		29.4 28.7
I_{11} R	71.4 57.7	42.2		
I_{11} L	73.5 58.8	42.5		
I_{12}	120.0	113.2 101.7	106.7	60.9
I_{13}	203.7	116.5 74.3		86.3 77.2
$\bar{x}$	122.5	105.3	81.1	63.8
SD	± 66.4	± 42.0	± 23.4	± 23.9
SEM	± 14.5	± 9.9	± 6.8	± 6.9

R_1-R_2, $P = > 0.15$

R_1-R_3, $P = < 0.025$

R_3-R_4, $P = < 0.05$

Table 3b. Renal Blood Flow - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	109.6 70.1	84.7 80.1	55.8	
D_2	152.7	110.1 75.2		122.0 92.9
D_3	145.5 138.9 85.5	145.7		97.2
D_4	135.9 108.6 104.4		92.4	96.7
D_5	137.9 152.8	159.7	172.0	103.3
D_6	184.5	202.2 124.5		159.6 104.9
D_7	127.1	156.5 87.0	84.1 130.7	
D_8	150.8	168.9 131.8	132.8	87.5

Table 3b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_9	199.5		97.4	228.6 118.5 86.9
D_{10}		101.0 71.2 55.6		57.5 46.3
$\bar{x}$	133.6	116.9	109.3	107.8
SD	± 34.4	± 42.8	± 38.4	± 45.8
SEM	± 8.9	± 11.1	± 14.5	± 12.7

R_1-R_3, $P = 0.08$

R_1-R_4, $P = 0.051$

R_3I-R_3D, $P = < 0.05$

R_4I-R_3D, $P = < 0.005$

I - Intact kidneys

D - Denervated kidneys

Table 4a. Renal Plasma Flow - Intact Kidneys

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	90.8	78.4	45.7 30.6	35.0
I_2	85.4	60.6	38.7 55.8	41.5
I_3	79.3	72.5	38.2 45.4	21.3
I_4		76.7 57.6	67.4	58.8 52.8
I_5	64.8 40.0 34.7			43.9 25.8
I_6	116.1 105.4	76.8	32.6	
I_7	173.8 121.5	109.3		
I_8	53.7	65.9 56.4	63.3 47.1	
I_9	105.4 94.4	73.3 69.8	57.4	

Table 4a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	61.3 35.9	31.7		14.0 17.2
I_{11} R	44.2 33.3	20.5		
I_{11} L	45.7 33.9	20.6		
I_{12}	53.4	59.9 56.4	50.0	31.1
I_{13}		128.3 73.5 47.8		47.4 48.1
$\bar{x}$	73.2	65.1	47.7	36.4
SD	± 28.6	± 26.2	± 11.2	± 14.6
SEM	± 6.4	± 6.0	± 3.2	± 4.2

R_1-R_3, $P = < 0.025$

R_1-R_4, $P = < 0.005$

Table 4b. Renal Plasma Flow - Denervated Kidneys

Expt. No.	R_1	R_2	R_3	R_4
	$PaCO_2$ 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	68.5 42.4	49.3 46.3	29.8	
D_2	85.3	56.9 45.2		51.3 44.2
D_3	80.0 74.2 43.2	72.4		42.6
D_4	83.6 67.9 67.3		58.6	51.0
D_5	88.9 89.7	95.2	94.4	52.9
D_6	97.2	99.4 61.3		76.1 47.2
D_7	70.1	82.7 51.7	43.7 71.1	
D_8	81.4	74.5 57.5	58.1	49.7

Table 4b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_9	106.5	44.1		94.7 49.9 38.5
D_{10}		60.7 42.9 33.1		29.7 26.1
$\bar{x}$	76.4	61.9	57.1	50.3
SD	±17.7	±19.3	±21.1	±17.2
SEM	± 4.6	± 5.0	± 8.0	± 4.8

R_1-R_2, $P = 0.05$

R_1-R_4, $P = < 0.005$

R_3I-R_3D, $P = > 0.10$

R_4I-R_4D, $P = < 0.025$

I - Intact Kidneys

D – Denervated Kidneys

Table 5a. Effective Renal Plasma Flow - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	$PaCO_2$ 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	56.7	51.1	28.7 22.0	20.7
I_2	51.5	38.7	26.9 32.2	17.2
I_3	44.5	42.2	21.3 32.4	14.0
I_4		49.7 40.7	44.9	35.4 34.8
I_5		44.2 27.9 24.7		28.8 16.3
I_6	73.8 64.9	46.4	20.0	
I_7	95.9 58.8	62.1		
I_8	41.6	44.9 42.8	43.6 33.5	
I_9	57.6 50.5	41.3 35.5	30.7	

Table 5a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	39.9	20.9		9.2
	18.7			10.7
I_{11}	27.7	12.1		
	20.4	13.2		
	28.7			
	21.1			
I_{12}	34.3	37.6	34.8	17.1
		36.6		
I_{13}		81.2		27.8
		52.2		30.4
		32.3		
I_{14} R	36.3	23.0	16.8	
	24.9	21.1		
$\bar{x}$	44.6	38.4	29.8	21.9
SD	±20.4	±15.7	± 8.7	± 9.1
SEM	± 4.6	± 3.2	± 2.4	± 2.6

R_1-R_3, $P = 0.01$

R_1-R_4, $P = < 0.005$

Table 5b. Effective Renal Plasma Flow - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	45.5 29.0	30.6 24.1	19.3	
D_2	59.5	43.6 35.1		36.2 30.0
D_3	64.2 59.2 34.1	55.3		30.3
D_4	51.6 41.3 41.5		30.3	29.3
D_5	64.6 65.2	57.0	53.7	43.3
D_6	58.0	63.8 38.1		50.4 29.5
D_7	45.2	41.1 29.7	24.7 38.9	
D_8	51.5	39.0 33.6	25.3	24.3
D_9	61.3		25.3	37.9 26.8 21.7

Table 5b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		31.7 24.0 20.2		17.9 12.8
$\bar{x}$	51.4	37.8	31.1	30.0
SD	±11.4	±12.7	±11.7	±10.2
SEM	± 2.9	± 3.3	± 4.4	± 2.8

R_1-R_2, $P = < 0.005$

R_2-R_3, $P = > 0.10$

R_1I-R_1D, $P = > 0.10$

R_4I-R_4D, $P = < 0.025$

I - Intact Kidneys

D - Denervated Kidneys

Table 6a. Glomerular Filtration Rate - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	17.8	21.2	17.3 15.8	14.6
I_2	18.8	16.1	13.8 14.8	8.8
I_3	17.8	19.4	12.5 20.1	10.1
I_4		28.0 31.1	27.2	24.2 24.9
I_5	20.3 17.5 16.0			15.8 11.9
I_6	32.0 29.6	21.4	11.8	
I_7	33.4 26.3	28.2		
I_8	17.8	23.3 25.8	25.5 22.9	
I_9	18.3 15.9	16.4 16.2	15.0	

Table 6a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	19.7 10.3	13.7		8.0 8.3
I_{11} R	8.3 8.0	7.9		
I_{11} L	8.7 7.3	6.8		
I_{12}	17.1	18.8 18.2	20.6	11.4
I_{13}		30.3 29.0 21.2		18.4 19.1
$\bar{x}$ SD SEM	18.0 ± 7.7 ± 1.7	20.7 ± 7.0 ± 1.6	18.1 ± 5.1 ± 1.5	14.6 ±6.0 ±1.7

R_1-R_4, P = 0.051

R_2-R_4, P = 0.005

Table 6b. Glomerular Filtration Rate - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	14.4 12.9	11.0 12.3	10.4	
D_2	17.6	16.9 16.1		17.2 13.0
D_3	24.1 20.7 15.9	22.8		16.0
D_4	25.1 18.1 20.7		16.0	16.9
D_5	27.5 27.2	32.7	25.2	24.6
D_6	18.2	17.9 21.7		24.1 18.9
D_7	18.8	18.1 13.5	14.7 15.2	
D_8	21.6	20.1 18.0	16.8	16.9
D_9	21.8		12.4	20.0 17.0 12.3

Table 6b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		18.7 15.9 14.7		13.1 10.3
$\bar{x}$	20.3	18.0	15.8	16.9
SD	± 4.4	± 5.2	± 4.7	± 4.3
SEM	± 1.1	± 1.4	± 1.8	± 1.2

R_1-R_3, $P = < 0.025$

R_1-R_4, $P = 0.025$

R_1I-R_1D, $P = 0.15$

R_4I-R_4D, $P = > 0.10$

I - Intact Kidneys

D - Denervated Kidneys

Table 7a. Filtration Fraction - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	0.197	0.275	0.378 0.158	0.418
I_2	0.220	0.265	0.359 0.265	0.261
I_3	0.224	0.265	0.340 0.443	0.479
I_4		0.366 0.541	0.403	0.412 0.473
I_5	0.313 0.438 0.462			0.360 0.462
I_6	0.275 0.281	0.278	0.362	
I_7	0.195 0.218	0.257		
I_8	0.333	0.365 0.459	0.396 0.487	
I_9	0.174 0.169	0.227 0.232		

Table 7a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	0.321 0.402	0.436		0.569 0.484
I_{11} R	0.187 0.238	0.387		
I_{11} L	0.195 0.227	0.331		
I_{12}	0.301	0.322 0.319	0.411	0.367
I_{13}		0.237 0.393 0.454		0.388 0.380

R_1-R_2, $P = < 0.025$

Table 7b. Filtration Fraction - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	0.211 0.305	0.223 0.267	0.351	
D_2	0.207	0.301 0.357		0.335 0.294
D_3	0.306 0.297 0.368	0.314		0.386
D_4	0.300 0.271 0.312		0.276	0.337
D_5	0.325 0.304	0.343	0.268	0.480
D_6	0.188	0.180 0.357		0.319 0.403
D_7	0.267	0.225 0.270	0.337 0.275	
D_8	0.265	0.275 0.313	0.290	0.314
D_9	0.205		0.280	0.241 0.341 0.328

Table 7b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		0.306 0.378 0.444		0.449 0.394
$\bar{x}$	0.274	0.303	0.296	0.357
SD	±0.052	±0.069	±0.039	±0.067
SEM	±0.013	±0.018	±0.010	±0.019

R_1-R_3, $P = 0.10$

R_2-R_4, $P = < 0.025$

R_2I-R_2D, $P = > 0.10$

R_3I-R_3D, $P = 0.074$

R_4I-R_4D, $P = < 0.025$

I - Intact Kidneys

D - Denervated Kidneys

Table 8a. Urine Flow - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_2	1.27	1.01	0.57 0.97	0.39
I_4		1.05 1.26	2.13	0.86 1.09
I_5	0.76 0.69 0.76			0.55 0.50
I_6	0.55 0.54	0.46	0.29	
I_7	0.93 0.73	0.46		
I_8	0.27	0.30 0.63	0.35 0.96	
I_9	1.43 1.69	1.16 1.39	1.11	
I_{10}	0.59 1.18	0.82		0.54 0.88
I_{11} L	1.76 2.05	1.01		
I_{12}	0.27	1.30 1.77	0.64	0.56

Table 8a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{13}		1.55 0.77 0.47		0.63 1.00
$\bar{x}$	0.87	0.87	0.71	0.55
SD	±0.46	±0.49	±0.50	±0.34
SEM	±0.09	±0.10	±0.13	±0.08

R_1-R_4, $P = < 0.025$

R_2-R_4, $P = < 0.01$

Table 8b. Urine Flow - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	0.80 0.91	1.27 0.94	0.65	
D_2	0.91	0.82 1.21		1.26 1.05
D_3	1.34 1.18 0.47	1.35		0.70
D_4	1.62 1.48 2.04		1.62	1.36
D_5	1.52 1.52	1.61	2.05	1.31
D_6	1.40	1.66 0.93		1.44 1.08
D_7	0.60	1.29 0.49	0.70 1.48	
D_8	0.97	1.39 1.86	1.61	1.87
D_9	1.16		0.48	0.60 0.58 0.51

Table 8b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		0.37 0.41 0.22		0.32 0.18
$\bar{x}$	1.19	1.05	1.23	0.94
SD	±0.42	±0.51	±0.61	±0.50
SEM	±0.11	±0.13	±0.23	±0.14

R_1-R_4, $P = > 0.10$

R_1I-R_1D, $P = < 0.025$

R_2I-R_2D, $P = > 0.10$

R_3I-R_3D, $P = 0.025$
R_4I-R_4D, $P = < 0.01$

I - Intact Kidneys

D - Denervated Kidneys

Table 9a. Osmolar Clearance - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	$\mathrm{Pa_{CO_2}}$ 25-45	$\mathrm{Pa_{CO_2}}$ 46-90	$\mathrm{Pa_{CO_2}}$ 91-130	$\mathrm{Pa_{CO_2}}$ 131-210
I_2	1.20	1.08	0.66 1.03	0.48
I_4		1.61 1.46	2.47	1.33 1.43
I_5	0.87 1.06 1.17			0.75 0.73
I_6	1.78 1.41	1.03	0.68	
I_7	1.95 1.82	1.21		
I_8	0.50	0.50 1.04	0.69 1.25	
I_9	2.01 1.91	1.46 1.32	0.93	
I_{10}	1.02 0.89	0.85		0.61 0.78
I_{11} L	1.56 1.47	0.89		
I_{12}	0.53	1.15 1.35	1.15	0.65
I_{13}		1.72 0.99 0.71		0.61 0.83

Table 9a. Continued.

Expt. No.		R_1	R_2	R_3	R_4
I_{15}	R	0.65 0.72 0.58		0.79	0.90
I_{16}	R	1.40 1.22	1.29	1.25	0.90
I_{17}	R	0.64	0.79 0.55		0.48
I_{18}	R	0.97	0.97 0.90	0.54 0.68	
I_{19}	R	0.77	0.65 1.37	0.71	1.12
I_{20}	R	0.92			0.14
I_{21}	R		0.55 0.78 0.66		0.38
$\bar{x}$		1.14	1.03	0.99	0.72
SD		±0.45	±0.34	±0.60	±0.37
SEM		±0.09	±0.07	±0.17	±0.09

R_1-R_4, P = $<$ 0.005

R_2-R_4, P = 0.005

R_3-R_4, P = 0.05

Table 9b. Osmolar Clearance - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	1.06 0.77	1.32 0.93	0.90	
D_2	0.84	0.82 1.11		1.33 1.61
D_3	1.75 1.45 0.73	1.89		0.61
D_4	1.05 1.34 1.18		1.28	1.23
D_5	1.47 1.86	1.64	2.44	1.66
D_6	0.86	1.19 0.87		1.41 0.98
D_7	1.17	1.60 0.76	0.91 1.38	
D_8	1.37	1.59 1.62	1.57	1.61
D_9	1.31		0.73	0.89 0.74 0.86
D_{10}		0.78 0.83 0.61		0.62 0.47
$\bar{x}$	1.21	1.17	1.32	1.08
SD	±0.34	±0.41	±0.58	±0.42
SEM	±0.09	±0.11	±0.22	±0.12

R_4I-R_4D, P = 0.01

Table 10a. Free Water Clearance - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_2	+0.07	-0.07	-0.07 -0.06	-0.09
I_4		-0.56 -0.20	-0.34	-0.47 -0.34
I_5	-0.13 -0.37 -0.41			-0.20 -0.23
I_6	-0.73 -0.87	-0.57	-0.39	
I_7	-1.03 -1.09	-0.76		
I_8	-0.23	-0.20 -0.41	-0.34 -0.29	
I_9	-0.58 -0.22	-0.30 +0.07	+0.18	
I_{10}	-0.043 +0.29	-0.03	+0.18	
I_{11} L	+0.20 +0.58	+0.12		
I_{12}	-0.26	+0.15 +0.42	-0.51	-0.09

Table 10a. Continued.

Expt. No.		R_1	R_2	R_3	R_4
I_{13}			-0.17 -0.22 -0.24		+0.02 +0.17
I_{15}	R	+0.34 -0.24 +0.31		-0.15	-0.46
I_{16}	R	-0.68 -0.66	-0.49	-0.50	-0.46
I_{17}	R	+0.28	+0.34 -0.12		-0.18
I_{18}	R	-0.50	-0.29 -0.36	-0.26 -0.13	
I_{19}	R	-0.30	-0.19 +0.11	-0.26 -0.13	
I_{20}	R	-0.26			-0.05
I_{21}	R		-0.33 -0.35 -0.40		-0.22
$\bar{x}$		-0.28	-0.18	-0.13	-0.14
SD		±0.45	±0.90	±0.82	±0.18
SEM		±0.09	±0.18	±0.23	±0.05

R_1-R_4, P = 0.10

R_3-R_4, P = 0.085

Table 10b. Free Water Clearance - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Paco_2 25-45	Paco_2 46-90	Paco_2 91-130	Paco_2 131-210
D_1	-0.26 +0.14	-0.05 +0.01	-0.25	
D_2	+0.07	0.00 +0.10		-0.07 -0.56
D_3	-0.42 -0.28 -0.27	-0.54		+0.09
D_4	+0.57 +0.14 +0.86		+0.34	+0.13
D_5	+0.05 -0.34	-0.03	-0.39	-0.35
D_6	+0.54	+0.47 +0.06		+0.03 +0.10
D_7	-0.57	-0.31 -0.27	-0.21 +0.10	
D_8	-0.40	-0.20 +0.24	+0.04	+0.26
D_9	-0.15		-0.25	-0.29 -0.16 -0.35
D_{10}		-0.48 -0.42 -0.39		-0.40 -0.29
$\bar{x}$	-0.02	-0.12	-0.89	-0.14
SD	±0.24	±0.28	±0.09	±0.79
SEM	±0.06	±0.07	±0.03	±0.22

R_1I-R_1D, $P = < 0.05$

R_3I-R_3D, $P = 0.09$

I - Intact Kidneys
D - Denervated Kidneys

Table 11a. Renal Resistance - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_2	1.00	1.16	2.32 1.51	2.41
I_3	0.98	0.99	1.80 1.70	3.73
I_4		0.99 1.56	1.17	1.13 1.45
I_5	1.20 2.35 2.73			1.38 2.65
I_6	0.72 0.77	0.80	2.31	
I_7	0.45 0.53	0.61		
I_8	1.31	0.91 1.12	1.01 1.39	
I_9	0.65 0.81	0.65 0.84	1.11	
I_{10}	1.01 1.50	1.38		3.57 2.96
I_{11} R	1.77 2.21	2.75		

Table 11a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{11} L	1.72 2.17	2.74		
I_{12}	0.72	0.94 1.09	0.95	2.06
I_{13}		0.70 1.25 1.95		1.65 1.74
$\bar{x}$	1.29	1.25	1.53	2.25
SD	±0.68	±0.64	±0.58	±0.89
SEM	±0.16	±0.15	±0.18	±0.27

R_1-R_4, P = < 0.005

R_1-R_3, P = > 0.10

R_2-R_3, P = > 0.10

Table 11b. Renal Resistance - Denervated Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	1.06 1.53	1.32 1.39	2.18	
D_2	0.78	1.15 1.62		0.89 1.28
D_3	0.84 0.96 1.57	0.78		1.38
D_4	0.88 0.97 1.10		1.19	1.03
D_5	0.94 0.85	0.82	0.73	1.39
D_6	0.54	0.62 0.85		0.65 1.19
D_7	0.71	0.55 1.29	1.33 0.78	
D_8	0.86	0.73 0.99	1.23	2.06
D_9	0.51		1.54	0.57 1.22 1.61

Table 11b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		1.00 1.80 2.31		2.06 2.98
D_{11}	1.05 0.72 0.92	0.64		0.64
$\bar{x}$	0.93	1.12	1.28	1.35
SD	±0.28	±0.49	±0.49	±0.66
SEM	±0.07	±0.12	±0.19	±0.18

R_1-R_2, $P = 0.085$

R_1I-R_1D, $P = < 0.025$

R_2I-R_2D, $P = > 0.15$

R_3I-R_3D, $P = > 0.15$

R_4I-R_4D, $P = < 0.005$

I - Intact Kidneys

D - Denervated Kidneys

Table 12a. Hematocrit - Intact Kidneys.

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
I_1	35.0	32.5	37.0 36.0	39.5
I_2	33.5	34.0	34.5 34.0	35.0
I_3	34.5	36.0	41.5 38.5	41.5
I_4		35.0 34.0	32.0	42.0 43.5
I_5	30.0 31.0 30.0			47.0 45.0
I_6	33.0 30.0	35.0	40.0	
I_7	39.5 52.0	52.0		
I_8	45.5	49.5 47.5	51.0 50.0	
I_9	36.5 35.0	44.5 39.0	45.0	

Table 12a. Continued.

Expt. No.	R_1	R_2	R_3	R_4
I_{10}	37.0	51.0		55.0
	43.5			42.0
I_{11} L	40.0	54.5		
	44.5			
I_{12}	58.5	49.5	56.0	51.5
		47.0		
I_{13}	39.0			47.5
		39.0		40.0
		38.0		
$\bar{x}$	38.3	42.2	41.3	44.1
SD	± 7.7	± 7.5	± 7.7	± 5.4
SEM	± 1.8	± 1.8	± 2.2	± 1.3

R_1-R_2, $P = 0.086$

R_1-R_3, $P = > 0.10$

R_1-R_4, $P = < 0.025$

Table 12b. Hematocrit - Denervated Kidneys

Expt. No.	R_1	R_2	R_3	R_4
	Pa_{CO_2} 25-45	Pa_{CO_2} 46-90	Pa_{CO_2} 91-130	Pa_{CO_2} 131-210
D_1	39.5 41.5	44.0 44.5	49.0	
D_2	46.0	50.0 41.5		61.0 55.0
D_3	47.5 49.0 53.0	53.0		61.5
D_4	40.5 39.5 37.5		38.5	50.0
D_5	37.5 43.5	42.5	47.5	51.5
D_6	49.5	53.5 53.5		55.0 58.0
D_7	46.5	49.5 48.0	50.5 43.0	
D_8	48.5	59.0 56.5	61.0	61.5
D_9	49.0		58.0	61.5 61.0 58.5

Table 12b. Continued.

Expt. No.	R_1	R_2	R_3	R_4
D_{10}		42.0 42.0 43.0		51.0 46.0
$\bar{x}$	44.6	48.2	49.6	56.3
SD	± 4.9	± 5.2	± 7.9	± 6.2
SEM	± 1.3	± 1.3	± 3.0	± 1.7

R_1-R_2, $P = < 0.05$

R_1-R_3, $P = < 0.025$

R_1-R_4, $P = < 0.005$

R_1I-R_1D, $P = 0.006$

R_2I-R_2D, $P = 0.006$

R_3I-R_3D, $P = < 0.025$

R_4I-R_4D, $P = < 0.005$

I - Intact Kidneys

D - Denervated Kidneys

APPENDIX I

During the first year of work on this project considerable effort was spent on refining and adapting techniques. Because it was realized that the best possible analytical results would be required to achieve the aims of the study, much attention was focussed on analytical methods (for PAH, Creatinine, etc.). During this period we became convinced that some of the work in the literature is open to question, especially where renal plasma flow has been estimated from PAH clearance. The kidney is particularly sensitive to loss of circulating blood volume, even that resulting from blood sampling, and this sensitivity is enhanced by barbiturate anesthesia. Surgical trauma during catheterization or cannulation procedures also leads to abnormal responses and extremely variable data.

One of the major problems encountered was that of obtaining reliable measurements of renal blood flow. We first tried to measure renal blood flow using the extraction and clearance of PAH. One of the first problems encountered was the difficulty in obtaining reproducible standard curves. This problem persisted as long as we used the method, and was such that we were never certain of the concentration being measured. At this time we made some direct

flow measurements by cannulating the renal vein and found that the discrepancy between directly and indirectly measured blood flow was so great that we lost all confidence in the PAH method. The reason behind our inability to make the PAH method work was never discovered. It has been shown that the use of I^{131}-Hippuran gives the same measurement as PAH (57) but has the advantage that no laborious chemical analysis is needed for its determination.

When we first started using I^{131}-Hippuran we obtained very low extraction ratios (0.1 to 0.4). From a report of Block and Burrows (112) who had encountered similar problems with I^{131}-Diodrast, it was concluded that when using only the radioisotope, the actual blood concentration of I^{131}-Hippuran is so low that transport across the renal tubule does not proceed in a normal manner. Infusing PAH along with the labelled Hippuran effected some improvement but extractions still remained low. The problem of extraction was finally solved by using unlabelled Hippuran as a carrier for the I^{131}-Hippuran. In order to prevent the diffusion of Hippuran from erythrocytes to plasma in the renal venous samples, the venous samples were taken in ice-cold syringes and separated in a refrigerated centrifuge within 1-2 minutes after drawing the sample. During the

5 minutes which elapsed between the time the sample was withdrawn, and the time the plasma was removed from the red cells, the sample was either in an ice-cold syringe, an ice-bath or in a refrigerated centrifuge.

With the I^{131}-Hippuran working well for the measurement of renal blood flow, we decided to do away with chemical techniques completely, and use C^{14}-Inulin for GFR measurement instead of creatinine. Samples were counted in a well scintillation counter, fitted with a radiation analyser which detected only I^{131}. An equivalent sample was then counted by a thin-window Geiger tube which detected both the I^{131} and the C^{14}. A computer program was written to separate the I^{131} and C^{14} counts. While the method was theoretically satisfactory, difficulties were encountered due to variation in sample absorption of C^{14} activity.

Another attempt was made to count the I^{131} and C^{14} using a liquid scintillation counter. Although the energy spectra of the two isotopes were too close for maximum resolution, this method would have worked. The sample preparation was so time consuming however (it included freeze-drying), that it was decided that nothing would be gained by using this more complicated method for analysis. We therefore reverted to the use of I^{131}-Hippuran for

the measurement of renal blood flow and creatinine for the measurement of GFR.

Attempts were made to control renal blood flow by perfusion of the kidney at predetermined flow rates via a segment of the aorta. In all experiments, techniques which had been used in our laboratory to successfully perfuse other organs led to a progressive increase in renal vascular resistance to very high values. This problem has been found to be a common one with perfusion of the intact kidney (9).

The next group of experiments was directed toward direct measurement of renal blood flow by cannulation of a renal vein, the collected outflow being returned to the circulation through a femoral vein after measurement. This, and a concurrent attempt to control arterial blood pressure using a cartesian manostat were also discarded, since even a brief interference with renal outflow led to cortico-medullary congestion to a degree which we considered unacceptable. Probably with enough time and practice, these direct measurement techniques could have been perfected.

It was finally decided that the most productive approach would be to minimize all surgical preparation. Barbiturate anesthesia (sodium thiopental) always led to a steady deterioration in the

animal, manifest by progressive fall in arterial blood pressure. We changed the anesthetic agent to α-chloralose with 1 mg/kg morphine as a premedication and in all subsequent experiments have found that despite considerable blood loss at times, the arterial pressure has been well maintained. This is an important consideration since in each experiment blood samples amounting to about 200 ml are drawn.

APPENDIX II

Abbreviations Used in the Text

RBF	Renal blood flow
RPF	Renal plasma flow
ERPF	Effective renal plasma flow
GFR	Glomerular filtration rate
FF	Filtration fraction
RR	Renal resistance
E_{Hipp}	Extraction of Hippuran
C_{Osm}	Osmolar clearance
C_{H_2O}	Free water clearance
Pa_{CO_2}	Arterial carbon dioxide tension
$C_{I_{CO_2}}$	Concentration of carbon dioxide in the inspired air

B29849